QMB Proteins Satellite: Abstracts and Posters

Speaker Abstracts P1-P26

P1

Innovation by Evolution: Expanding the Enzyme Universe

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Not satisfied with nature's vast catalytic repertoire, we want to create new enzymes to expand the range of genetically encoded chemistry. I will describe how we can use the most powerful algorithm for biological design, evolution, to optimize existing biocatalysts and create whole new ones. Mimicking nature's evolutionary tricks and using a little chemical intuition, we can generate whole new enzyme families that catalyze important reactions not (yet) known in nature.

P2

Discovery, engineering and applications of non-ribosomal peptide synthetase and phosphopantetheinyl transferase enzymes

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Non-ribosomal peptide synthetases (NRPS) are enzymes that generate small peptide products independent of the ribosome, a mechanism that enables incorporation of unusual amino acid substrates (over 500 known!). NRPS typically have a multi-modular structure, where each distinct module governs incorporation of a specific amino acid substrate into the peptide product. To achieve this, individual modules are comprised of discrete domains that play key roles in substrate recognition, activation, translocation and condensation.

For an NRPS module to be functional its peptidyl carrier protein (PCP) domain must first be recognized and activated by an enzyme partner known as a phosphopantetheinyl transferase (PPTase), which attaches a flexible prosthesis derived from coenzyme A. This then acts as a "swinging arm" to transfer intermediates between modules. We have exploited this requirement to recover novel PPTase genes from environmental DNA libraries, using reporter strains of *E. coli* that express an unusual single-module NRPS named BpsA. Following activation by a PPTase, BpsA is able to condense two molecules of L-glutamine into a blue pigmented product, indigoidine – resulting in colonies that are easily detected on agar plates. We have shown that approximately 50% of PPTase genes are part of a secondary metabolite biosynthetic cluster, and that BpsA can be used to locate these gene clusters. Moreover, by replacing the native PCP-domain of BpsA with alternative PCP-domains (followed by a bit of "massaging" via error-prone PCR mutagenesis to restore function) we found we could recover additional PPTases that did not recognise native BpsA.

PPTases are also useful in biotechnology and nanotechnology, as many of them can use modified analogues of coenzyme A that contain fluorescent or click chemistry capable substituents. Basically, they are handy for attaching all kinds of things to other things via a wiggly tether. We have used directed evolution with a combined positive/negative screening strategy to modify the *E. coli* PPTase EntD from having a broad specificity for PCP-domains in general, to being much more specific for certain PCP-domains. Our aim is ultimately to engineer pairs of PPTases that have non-overlapping specificities for different attachment sequences ('tags'), to enable site-specific labeling of differentially tagged proteins in a complex mixture. We figure that capability will be useful to someone, somewhere, someday.

P3

Protease-based synthetic sensing and signal amplification

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The bottom-up design of protein-based signaling networks is a key goal of synthetic biology; yet, it remains elusive due to our inability to tailor-make signal transducers and receptors that can be readily compiled into defined signaling networks. Here, we report a generic approach for the construction of protein-based molecular switches based on artficially autoinhibited proteases. Using structure-guided design and directed protein evolution, we created signal transducers based on artificially autoinhibited proteases that can be activated following site-specific proteolysis and also demonstrate the modular design of an allosterically regulated protease receptor following recombination with an affinity clamp peptide receptor. Notably, the receptor's mode of action can be varied from >5-fold switch-OFF to >30-fold switch-ON solely by changing the length of the connecting linkers, demonstrating a high functional plasticity not previously observed in naturally occurring receptor systems. We also create an integrated signaling circuit based on two orthogonal autoinhibited protease units that can propagate and amplify molecular queues generated by the protease receptor. Finally, we present a generic two-component receptor architecture based on proximity-based activation of two autoinhibited proteases. Overall, the approach allows the design of protease-based signaling networks that, in principle, can be connected to any biological process.

P4

Bio-inspired tectons: Lsm protein rings as RNA-responsive nano-scaffolds

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The potential for protein tectons in nanotechnology is increasingly recognized, but the repertoire of stable proteins that form defined shapes in response to a specific trigger is limited. We have exploited the self-assembling capacity of Lsm proteins, which form ring scaffolds (as 6- or 7-component oligomers) to gather up RNA and protein partners for their cellular role in RNA regulation. Tubule or ring-forming proteins are appealing as tectons for construction of nanowires, as nanocapsules for molecular transport, or as scaffolds for evolution of two-dimensional arrays.

We will outline our repertoire of recombinant Lsm ring tectons, which incorporates highly stable modules derived from thermophilic organisms, as well as fused polyprotein systems with simplified symmetry. These ring tectons are able to bind specific RNA sequences, and can be manipulated to alter their internal pore dimensions. Through supramolecular engineering (modifications with Cys and His moieties), we have successfully fabricated Lsm rings into metal-dependent cage-like clusters, as well as redox-sensitive tubule stacks. Our molecular views of these constructions come from electron microscopy as well as crystallography. These new responsive Lsm materials offer exciting possibilities for applications as RNA delivery capsules, or biosensors.

P5

Intrinsically Disordered Protein and the Origins of Complex Multicellular Organisms

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DNA sequencing has enabled the widespread construction of phylogenetic trees, revealing that *multicellular* organisms evolved independently from unicellular ancestors about 25 times among prokaryotes and eukaryotes. Multicellular organisms can be classified as simple, in which all of the cells are in direct contact with the surrounding milieu, or complex, in which some cells are completely surrounded by other cells. Current phylogenetic trees indicate that *complex multicellular* organisms evolved independently from unicellular ancestors about 10 times, and only among the eukaryotes, including once for animals, twice each for green, red, and brown algae, and thrice for fungi.

Given these multiple independent evolutionary lineages, we asked two questions: 1. Which molecular functions underpinned the evolution of multicellular organisms?; and, 2. Which of these molecular functions depend on intrinsically disordered proteins (IDPs, reviewed in [1])? Multicellular organisms require the advent of molecules for cellular adhesion, for cell-cell communication and for developmental programs. In addition, the developmental programs need to be regulated over space and time. Finally, each multicellular organism has cell-specific biochemistry. As for dependence on IDPs, we ranked Swiss Protein key-words with regard to their degree of association with predictions of protein structure or disorder. With a Z-sore of 18.8 compared to random-function proteins, “differentiation” was the biological process most strongly associated with IDP predictions. As expected from this result, large numbers of individual proteins associated with differentiation exhibit substantial regions of predicted disorder [2]. All five of the underpinning molecular functions for multicellularity were found to depend critically on IDP-based mechanisms [3].

These new findings necessitate a rethinking of the gene regulatory network models currently used to explain cellular differentiation and the evolution of complex multicellular organisms [4].

P6

Binding of fullerenes and nanotubes to the mechanosensitive channel of large conductance: A possible new class of antimicrobials

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Multi-drug resistance is becoming an increasing problem in the treatment of bacterial infections and diseases. For example, the re-emergence of tuberculosis is a serious threat that is spreading rapidly throughout the world. It has become increasingly important to develop new antibiotics to combat the rapidly emerging strains of multiple drug-resistant bacterial strains. A potential target for new antibiotics is the mechanosensitive channel of large conductance (MscL) which is highly conserved among prokaryotes.[1] MscL channels act as emergency release valves that open in response to membrane tension caused by hypo-osmotic shock. They relieve pressure and therefore prevent membrane damage. Evidence suggests that a pharmacological agent that can affect the gating of, or block the current through, MscL has significant potential as a new class of antimicrobial compounds[1] capable of targeting a range of pathogenic bacteria with minimal side-effects to infected patients.

Using molecular dynamics simulations we examine the binding of fullerenes and nanotubes to MscL and demonstrate that both are stable within the MscL pore.[2] We predict that fullerenes will attenuate the flow of ions through MscL by reducing the pore volume available to water and ions resulting in a slower recovery from osmotic downshock. However, nanotubes will prevent pore closure resulting in a permanently open pore, thus causing the cell to continually leak cytoplasmic contents. Both of these events may be sufficient to cause a bacterial cell to lyse. Moreover, we confirm experimentally that it is possible to attenuate the flow of ions through MscL using a C_{60}-γ cyclodextrin complex.[2]

1. Booth, I.R. and P. Blount (2012). *The MscS and MscL families of mechanosensitive channels act as microbial emergency release valves.* Journal of Bacteriology. 194: 4802-4809.
2. Hilder, T.A., P. Ridone, Y. Nakayama, B. Martinac and S.H. Chung (2014). *Binding of fullerenes and nanotubes to MscL.* Scientific Reports. 4: 5609.

P7

Multiscale Dynamics of the Dengue Virus Envelope

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Dengue is a pathogenic flavivirus, consisting of a nucleocapsid core surrounded by a lipid bilayer membrane plus anchored envelope (E) and membrane (M) proteins. Infection rearrangement of the E protein, exposing the unstructured fusion peptide (FP) for viral/host membrane fusion. Currently there are no available vaccines effective against all Dengue serotypes, due largely to the dynamic nature of the E protein. A multiscale simulation approach has thus been adopted to characterize the dynamics of the E protein in the context of fusion and viral architecture. We first report a systematic comparison of simulation methods and forcefields, constituting ~50 µs of sampling, to determine the complete conformational landscape of FP across all known flavivirus family members. The solvated peptide fold was significantly less ordered than in the crystallographic state, confirmed via circular dichroism and spectrofluorometric measurements, and the conformational landscape of a non-fusogenic mutant was perturbed compared to wild-type variants, emphasizing the importance of dynamics in therapeutic targeting. Subsequently, long-timescale FP/membrane assembly was investigated, helping to refine previously derived solution NMR data, and atomic-resolution free energy profiles calculated. FP binding affinities validated by fluorescence spectroscopy revealed functionally important differences between Dengue serotypes, and a reduced interaction (>50%) with pure zwitterionic membranes compared to physiologically realistic endosomal membranes, highlighting the importance of electrostatics in virus-host recognition. Finally, we reconstructed the entire Dengue viral envelope complex, containing 180 copies each of the M and E proteins within a ~40 nm lipid bilayer vesicle. Microsecond-timescale simulations revealed that the E protein induces extreme local curvature and lipid sorting, which may be important during membrane fusion. Calculation of theoretical, time-dependent density maps revealed almost perfect agreement with the equivalent density from low-resolution cryo-EM data. This is being further extended by integrating a geometrically plausible model of the viral core containing the entire viral genome.

P8

Assembly of a cell-specific transcription factor complex

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The up- or-down-regulation of specific genes is governed by combinations of cell specific transcription factors that bind to promoter and enhancer elements, remodel chromatin, trigger chromatin looping, and recruit the basal transcription machinery. We have used a range of structural biology and biophysical approaches to gain a more complete understanding of how a set of transcription factors come together to form the pentameric complex (comprising GATA1, LMO2, LDB1, Tal1 and E2A proteins), which regulates gene expression during blood cell development.

Although several components from this complex bind strongly to each other, others bind weakly, use intrinsically disordered binding domains, or are aggregation prone in isolation, meaning that we have had to implement a tethering approach to generate stable complexes for structure determination, and develop novel approaches to determine binding affinities. Our data are providing new insights into the roles of strong versus weak interactions and the recruitment of accessory proteins in the formation of cell-specific transcription complexes.

P9

Substrate Recognition by the Kinases and Phosphatases that Modify Translation Factor eIF2

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Phosphorylation of translation factor eIF2α on Ser51 is a common mechanism to regulate general and gene-specific mRNA translation. Four eIF2α kinases, PKR, GCN2, PERK and HRI, are activated under distinct cellular stress conditions. Activation of these kinases involves back-to-back dimerization of the kinase domains leading to kinase autophosphorylation and substrate recognition. The OB-fold domain at the N-terminus of eIF2α docks on helix αG in the C-terminal lobe of the kinase to position Ser51 near the active site of the kinase. Comparison of the structure of free eIF2α with the structure of the eIF2α–PKR complex revealed that Ser51 must undergo substantial repositioning to access the catalytic site of the kinase. Using molecular genetic and structural techniques we found that upon docking to PKR, eIF2α undergoes a conformational change to expose Ser51. This requirement for an induced conformational change in eIF2α prevents promiscuous phosphorylation by heterologous kinases and restricts Ser51 phosphorylation to the family of eIF2α kinases. Upon relief of the cellular stress signal, the kinase is deactivated and protein phosphatase 1 (PP1) dephosphorylates Ser51. In contrast to the exquisite substrate specificity of the eIF2α kinases, PP1 dephosphorylates a broad range of substrates. PP1 regulatory subunits target the phosphatase to its specific substrates. In mammals the proteins GADD34 and CReP target PP1 to dephosphorylate eIF2α. We found that in yeast, which lack GADD34 and CReP, the eIF2γ subunit targets PP1 to dephosphorylate eIF2α. Interestingly, mammalian GADD34, as well as several structurally related viral proteins, can functionally substitute for the PP1-binding site in eIF2γ and promote eIF2α dephosphorylation in yeast. These PP1 targeting proteins share a common 19 amino acid motif that directly binds to eIF2α. We propose that GADD34 and its related viral orthologs direct specific dephosphorylation of eIF2α by interacting with both PP1 and eIF2α through independent binding motifs.

P10

Structural basis of protein cargo transport by sorting nexin 27 and the retromer complex sorting machinery

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Sorting nexin (SNX) proteins are a large family of proteins with essential functions in the spatial and temporal regulation of cell trafficking and signaling in the endosomal membrane network. SNX27 is the only member of this family that contains a PSD95, Dlg1, ZO-1 (PDZ) interaction module, which in cooperation with the heterotrimeric retromer complex, is a major regulator of endosome-to-plasma membrane recycling of transmembrane cargos that contain a PDZ-binding motif (PDZbm).

Here we describe the previously unexplored core interaction in SNX27-retromer assembly and its functional relevance for cargo sorting. Crystal structures and NMR experiments reveal (i) an exposed β-hairpin in the SNX27 PDZ domain engaging a groove in the arrestin-like structure of the vacuolar protein sorting 26A (VPS26A) retromer subunit[2] and (ii) establish how the SNX27 PDZ domain simultaneously binds PDZbms and retromer-associated VPS26. By further exploring the structural features of cargo binding by SNX27 we identified specific acidic amino acid sequences upstream of the PDZ triplet required for high affinity engagement of the SNX27 PDZ domain[3]. However, a subset of SNX27 ligands such as the β_2-adrenergic and N-methyl-D-aspartate receptors lack these molecular determinants. Instead we identify conserved sites of serine and threonine phosphorylation that substitute for acidic residues, and dramatically enhance SNX27 interactions. This newly identified mechanism provides a regulatory switch for PDZ interaction and protein transport. Defining the SNX27-binding code allowed us to distinguish and classify more than four hundred potential SNX27 ligands with broad functional implications in signal transduction, trafficking, neuronal development and cell adhesion.

With disruption of SNX27 and retromer function linked to synaptic dysfunction and neurodegenerative disease, our work provides the first step in the molecular description of this important sorting complex, and more broadly describes a unique tripartite interaction between a PDZ domain, an arrestin-like fold and a phosphorylated sorting motif.

1. Gallon, M.*, Clairfeuille, T.* *et al.* (2014) *A unique PDZ domain and arrestin-like fold interaction reveals mechanistic details of endocytic recycling by SNX27-retromer.* PNAS 111(35): E3604-13.
2. Clairfeuille, T. *et al.* (2015) *An electrostatic code and phosphorylation of PDZ binding motifs control cargo recognition by the SNX27-retromer endosomal recycling complex.* In preparation.

P11

Molecular mechanism of CCAAT-Enhancer Binding Protein (C/EBP) recruitment by the Trib1 pseudokinase

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The CCAAT-enhancer binding proteins (C/EBPs) are a family of transcription factors that play a central role in differentiation and proliferation of myeloid cells and adipocytes. Tribbles pseudokinases (Trib1 and Trib2) govern the cellular balance of C/EBPs by recruiting them to the COP1 ubiquitin E3 ligase for ubiquitination. Here we present the first crystal structure of a Tribbles protein, which reveals a catalytically-inactive Trib1 pseudokinase domain. Trib1 shows a unique adaptation in the αC-helix, an element central to regulation of many *bona fide* kinases. A second crystal structure and biophysical studies of the Trib1 kinase-like domain with its C-terminal extension, which includes the COP1-binding motif, show that the C-terminal extension is sequestered at a site formed by the modified Trib1 αC-helix. Additionally, we have identified and characterised the Trib-recognition sequence within C/EBP family members, and show that Trib1 recruits C/EBPα and C/EBPε more avidly than C/EBPβ or C/EBPδ. Further binding studies show that the Trib1 COP1-binding motif impedes recruitment of C/EBPs. This suggests that Trib1 exists in an autoinhibited state until C/EBPs displace the COP1 binding motif for recruitment to the COP1 E3-ligase. Together, this demonstrates a new molecular mechanism of pseudokinase regulation of signalling complex assembly, which has important implications for both myeloid cell development and malignancies.

P12

The mycobacterial PhoH2 proteins are type II toxin antitoxins coupled to RNA helicase domains

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PhoH2 proteins are found in a diverse range of organisms that span the bacterial tree and little is known about this large protein family. PhoH2 proteins have two domains: An N-terminal PIN domain fused to a C-terminal PhoH domain. The genome of *Mycobacterium tuberculosis* encodes 48 PIN domains and 47 of these constitute the VapC components of the 47 VapBC toxin-antitoxins. The 48th member of the *M. tuberculosis* PIN domain array is found in the single PhoH2 protein encoded in the genome. All characterized PIN domain proteins are RNases and the PhoH domains are predicted ATPases. This fusion of a PIN domain with an ATPase reflects a much wider association between PIN domains and PhoH domains across many prokaryote genomes. Here, we examine PhoH2 proteins from *M. tuberculosis*, *M. smegmatis* and a thermophilic homologue from *Thermobispora bispora* and we show that PhoH2 is a sequence specific RNA helicase and RNAse. In addition, phoH2 from M. tuberculosis and *M. smegmatis* is part of a longer mRNA transcript which includes a small, unannotated open reading frame (ORF) upstream of the phoH2 gene. This small gene overlaps with the beginning of the phoH2 gene in a manner similar to the PIN domain toxin antitoxin operons. We have annotated the upstream gene as phoAT and its putative promoter elements satisfy previously characterized consensus sequences at the -10 site. Conditional growth experiments carried out in M. smegmatis revealed a negative effect on growth by the expression of M. tuberculosis PhoH2 that was alleviated by co-expression of the PhoAT peptide. Thus in *M. tuberculosis*, PhoH2 represents a new variation on a type II PIN domain toxin antitoxin systems such that the toxin-antitoxin is now coupled to an RNA helicase whose predicted biological function is to unwind and cleave RNA in a sequence specific manner.

P13

Developing potent and specific inhibitors of the Grb7 breast cancer target using phosphotyrosine mimetics and bicyclic peptides

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Signalling pathways governing proliferation and migration are frequently deregulated in cancerous cells and are an attractive target for the development of novel therapeutics. Growth factor receptor bound protein 7 (Grb7) is an intracellular adaptor protein with an established role in these signalling processes. Due to the role of Grb7 and its overexpression in an abundance of cancers, Grb7 has been identified as an exciting and opportune therapeutic target.

The non-phosphorylated cyclic peptide, G7-18NATE, binds to the Grb7 SH2 domain, blocking Grb7 interactions with its upstream binding partners. Cell permeable G7-18NATE has been shown to inhibit pancreatic cell migration as well as reducing cellular growth and migration in breast cancer cell lines. The peptide binds to Grb7 with moderate affinity (18 µM); therefore, to improve on this, derivatives have been developed and tested using a structure-based drug design approach. G7-18NATE derivatives with either a carboxymethylphenylalanine (M1) or caboxyphenylalanine (M2) substitution have 3-fold and 9-fold increased affinity for the Grb7 SH2 domain compared with G7-18NATE, respectively. The X-ray crystal structure of the Grb7 SH2: M1 complex clearly reveals additional protein-peptide interactions occurring in the Grb7 phosphotyrosine binding pocket[4]. Bicyclic derivatives with varying additional linkages produce nM affinity with the structural analysis identifying unexpected modes of binding occurring at an alternate interface to the phosphotyrosine mimetic.

Combining both these strategies, and hence covering a broad Grb7 interface, has produced a potent inhibitor of Grb7 (K_D = 380 nM) and marks considerable progress in Grb7 based anti-cancer drug development. Developing these G7-18NATE derivatives will establish fundamental methods that can be readily applied to other intracellular therapeutic targets.

P14

High resolution cryo-electron microscopy structure of human peroxiredoxin-3 filament reveals the assembly of a putative chaperone

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Peroxiredoxins (Prxs) are a ubiquitous class of thiol-dependent peroxidases that play an important role in the protection and response of cells to oxidative stress. The catalytic unit of typical 2-Cys Prxs are homodimers, which can self-associate to form complex assemblies that are hypothesized to have signaling and chaperone activity. Mitochondrial Prx3 forms dodecameric toroids, which can further stack to form filaments, the so-called high-molecular-weight (HMW) form that has putative holdase activity. We used single-particle analysis and helical processing of electron cryomicroscopy images of human Prx3 filaments induced by low pH to generate a ~7-Å resolution 3D structure of the HMW form, the first such structure for a 2-Cys Prx. The pseudo-atomic model reveals interactions that promote the stacking of the toroids and shows that unlike previously reported data, the structure can accommodate a partially folded C terminus. The HMW filament lumen displays hydrophobic patches, which we hypothesize bestow holdase activity.

P15

Mass spectrometry of membrane proteins - the lipid connection

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The realisation that the lipid environment is crucial for maintaining the structure and function of membrane proteins prompts new methods to understand lipid interactions. One such method, mass spectrometry, is emerging with the potential to monitor different modes of lipid binding to membrane protein complexes. Initial studies monitored the addition of lipids and deduced the kinetic and thermodynamic effects of lipid binding to proteins. Recently however, we have focused on identifying lipids already present, explicitly in plugs, annular rings or cavities. Lipids that bind within these orifices to membrane proteins will have higher residence times than those in the bulk lipid bilayer and consequently can be quantified and characterized by mass spectrometry. In special cases, lipids identified within cavities have been proposed as substrates following activity assays. Alternatively, a gas phase unfolding protocol can be used to distinguish lipids that are important for stability. In this lecture I will provide an overview of recent advances in mass spectrometry, with a particular focus on the distinction of the various modes of lipid binding, their implications for structure and function, as well as new directions that lie ahead.

P16

Understanding multidrug transporter function: can substrate specificity and promiscuity co-exist?

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Two key characteristics of the multidrug transporter P-glycoprotein are its substrate promiscuity and structural flexibility. P-glycoprotein transports over 200 endogenous and drug substrates and plays a central role in the development of multidrug resistance in cancer. While residues essential for transport and binding have been identified, the large-scale structural rearrangements that occur during its transport cycle have made it difficult to pinpoint the physical location, exact molecular composition and specificity of the any potential substrate binding site. Here molecular dynamics simulations are used to calculate the free energy profile for the binding of morphine, nicardipine Hoechst 33342, Rhodamine 123, paclitaxel, tariquidar and verapamil to P-glycoprotein. We show that morphine and nicardipine primarily interact with key residues implicated in binding and transport from mutational studies, binding at different but overlapping sites within the transmembrane pore. Furthermore, their permeation pathways were distinct, but involved overlapping sets of residues. The results indicate that the binding location and permeation pathways of morphine and nicardipine are not well separated and cannot be considered as unique. Similar trends are identified for other compounds investigated, allowing us to propose structural mechanisms for competitive and non-competitive binding of the substrates investigated. Our results are consistent with a range of experimental studies and provide a structural perspective for our understanding of substrate uptake and transport by P-glycoprotein.

P17

Investigating the transition of a three-helix bundle from the molten globule to the fully-folded state

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One of the fundamental unknowns in biology is the pathway by which proteins fold to attain a unique three-dimensional structure. We are studying protein folding using a small domain from the RNA polymerase of paramyxoviruses. This family of viruses includes human pathogens such as mumps, measles and parainfluenza viruses. The RNA genomes of paramyxoviruses are packaged with protein to form a filamentous structure called the nucleocapsid. During replication the viral RNA polymerase 'walks' along the nucleocapsid. The domain we are studying acts as the feet of the polymerase, reversibly binding and releasing the nucleocapsid as the polymerase translocates. Previously we have shown that, although it appears that the foot domains of all paramyxoviruses fold into a three-helix bundle upon nucleocapsid binding, in solution some are structurally disordered while others are high ordered[1,2,3]. This reflects a delicate balance between order and disorder for the foot domains, and provides us with a natural laboratory for investigating protein folding

Currently we are examining the mumps virus foot domain, which exists as a molten globule in solution, with significant helical secondary structure but lacking stable tertiary contacts[2,3]. We can induce this domain to fold into an ordered three-helix bundle through addition of the protein stabiliser trimethylamine *N*-oxide (TMAO). We are using a range of biophysical techniques to examine the sequence of events that occur as the mumps virus foot domain folds. In particular, using NMR spectroscopy we aim to detect the point at which the nine residues at the hydrophobic core of the protein lock into place, and to relate this to the global contraction in size, and the stabilization of the helical backbone, which also occur during the transition. Our initial data suggests that there is a distinct break-point in the transition from the molten globule to the ordered state.

1. Kingston, R. L., Baase, W. A. & Gay, L. S. *Characterization of nucleocapsid binding by the measles virus and mumps virus phosphoproteins.* Journal of Virology **78**, 8630–8640 (2004).
2. Kingston, R. L., Gay, L. S., Baase, W. S. & Matthews, B. W. *Structure of the nucleocapsid-binding domain from the mumps virus polymerase; an example of protein folding induced by crystallization.* Journal of Molecular Biology **379**, 719–731 (2008).
3. Yegambaram, K., Bulloch, E. M. M. & Kingston, R. L. *Protein domain definition should allow for conditional disorder.* Protein Science **22**, 1502–1518 (2013).

P18

T cell receptor recognition of CD1a presenting self-lipid antigens

Birkinshaw, R.W.[1], Pellicci, D.G.[2], Cheng, T.3, Keller, A.N.[1], Sandoval-Romero, M.[1], Gras, S.[1], de Jong, A.[4], Uldrich, A.P.[2], Moody, D.B.[3], Godfrey, D.I.[2] and Rossjohn, J.[1]

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The Immune system is our principle defense in a constant battle against pathogenic organisms. A fundamental process in our ability to mount a successful immune response is the ability of T lymphocytes (T cells) to survey our bodies for signs of infection. Key to a successful T cell response is recognition of a suitable foreign antigen by the T cell receptor (TCR) and initiation of a signaling cascade that sacrifices the infected cell to prevent further pathogenic spread.

Classical antigen presentation is performed by the major histocompatibity complexes (MHC), presenting short peptide sequences to the T cell receptor for immune surveillance. More recently it has become apparent that in addition to peptides, lipid molecules can be presented to T cells via the CD1 family. CD1a presents a broad repertoire of lipid-based antigens including many host/self-derived lipids. This talk will discuss our recent work unraveling the antigen specificity of a CD1a restricted TCR and obtaining a molecular insight into this recognition thought the first crystal structures of a CD1a—TCR complex1. The study revealed that TCR was reactive to permissive endogenous antigens, while other lipid antigens were non-permissive to TCR binding. The structures of two TCR—CD1a-lipid complexes showed that the TCR recognized the surface of CD1a in a manner that precluded direct contact with permissive antigens. Non-permissive lipids indirectly inhibited TCR binding by disrupting the TCR—CD1a contact zone.

In determining the structures of ternary TCR–CD1 lipid complexes, we have shown that CD1a autoreactivity can result from direct contact of the TCR with CD1a itself, but only when the surface of CD1a is not disrupted by non-permissive ligands. This represents a previously unknown form of antigen detection in the immune system.

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P19

Reconstructed Ancestral Enzymes and Enzyme Evolution *in vivo*

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Ancestral sequence reconstruction (ASR) has been widely used to study historical enzyme evolution, both from a biochemical and cellular perspective. We have used ASR to reconstruct a range of ancestral enzymes that belong to key metabolic pathways in bacteria [1]. One of the most surprising findings from our work on the evolution of isopropyl malate dehydrogenase (IPMDH), for example [2], is that the ancestral enzyme (from the last common ancestor of Bacillus) is catalytically faster by a factor of 10 and kinetically more stable by a factor of 30, when compared to any known contemporary Bacillus IPMDH enzyme (including contemporary thermophilic IPMDHs). The ancient IPMDH is something of a 'super-enzyme' and this begs the question: Why do modern day Bacillus species no longer have such fast and stable enzymes? In this individual case, it is clear that catalytic speed and kinetic stability are not the only parameters that are being optimised during the course of evolution. We have improved our ASR approaches and we have replaced contemporary enzymes with their ancestral counterparts in modern bacteria to assess fitness in the context of organism growth. Our hypothesis is that neither the optimisation of enzyme rates, nor stability, is necessarily the dominant factor in the course of organismal evolution.

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P20

Functional and structural analysis of the transcriptional repressor KstR from *Mycobacterium tuberculosis*

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One of the hallmarks of the human pathogen *Mycobacterium tuberculosis* (*Mtb*) is its ability to persist in the severe environment inside phagosomes in the activated macrophage. It has been suggested that *Mtb* switches its metabolism to favour cholesterol utilization in order to survive in the nutrient-poor phagosome after INF-γ activation1. KstR is a highly conserved TetR-family transcriptional repressor that regulates a large subset of genes taking part in the degradation of cholesterol and fatty acids2. In agreement with the proposed importance of these carbon sources, many genes in this regulon including *kstR* itself are induced during infection of various models and/or are essential for survival of *Mtb in vivo*2, suggesting a role for this regulator in *Mtb*'s adaptation to the intracellular lifestyle that defines its pathogenesis. Knowledge of KstR function is therefore useful information for targeting cholesterol metabolism in the search for a new drug that would be synergistic with current therapy.

In this study we identify two ligands of KstR, both of which are CoA thioester derivatives from β-oxidation of the cholesterol aliphatic side chain. We confirm ligand binding using instrinsic fluorescence quenching, and we see a strong inhibitory effect on KstR-DNA binding using surface plasmon resonance, with IC50 as low as 25 nM of ligand. Crystal structures of apo-KstR show flexibility in the positions of the DNA-binding domain (DBD), which adopts variable positions in two different crystal forms. In constrast, structures of KstR-ligand complexes are highly similar to each other, despite different ligand affinities and demonstrate a seperation of the DBD that is unfavourable for DNA-binding. Although the position of the CoA moiety cannot be located in these complexes, comparison of ligand-bound and ligand- free structures highlights potential residues involved in ligand specificity and in the induction of conformational change upon ligand binding.

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P21

Characterization and engineering of the diol dehydratase from *Clostridium autoethanogenum*

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Glycerol and diol dehydratases are remarkable enzymes that use radical catalysis to remove water molecules from diols, effectively creating a carbonyl carbon from two adjacent alcohol functional groups. Such behaviour expands the repertoire of chemical transformations attainable in certain molecules, making these enzymes valuable tools for synthetic biology.

The diol dehydratase from *Clostridium autoethanogenum* (*Ca*PDHt) and its putative activating enzyme (*Ca*AE) have been suggested to be involved in the conversion of (*R*)-1,2-propanediol and (*S*)-1,2-propanediol to acetone and propanal *in vivo*, respectively[1]. The unusual conversion of different isomers of the same molecule into two different products implies a reaction mechanism that may involve functional and structural flexibility, making *Ca*PDHt suitable for engineering.

The *Ca*PDHt enzyme shares sequence homology with a previously characterized glycerol dehydratase from *Clostridium butyricum*, whereas a BLAST sequence alignment suggests that the *Ca*AE belongs to the glycyl-radical enzyme (GRE) activating proteins[2]. Therefore, we hypothesized that the *Ca*PDHt was likely to be a strictly anaerobic GRE protein, activated by a radical generated by the *Ca*AE. *Ca*PDHt and *Ca*AE are of further interest because *C. autoethanogenum* natively produces a diol, (*2R,3R*)-butanediol[3]. Effective dehydration of this molecule would result in a biological route to butanone. However, the wild type *Ca*PDHt does not show activity towards this butanediol isomer *in vivo*. Our goal is to engineer *Ca*PDHt variants that are able to dehydrate this molecule.

In this study, we describe the activity, selectivity and mechanism of *Ca*PDHt and *Ca*AE. We demonstrate the ability to express them in *E. coli* and purify aerobically, followed by activation under strict anaerobic conditions. We also present preliminary results from semi-rational design approaches to modify *Ca*PDHt substrate selectivity towards (*2R,3R*)- butanediol, and the development of suitable screens for mutants.

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P22: Exposing hidden allostery

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Allosteric regulation of protein function is important for metabolic control. The traditional understanding of allostery centres around conformational changes associated with ligand binding. However, more recently, examples of allostery without conformational change have been identified and the role of protein dynamics, intrinsic disorder and local unfolding has been shown to be important in the allosteric response. Understanding the molecular detail of the way in which the allosteric signal is transmitted in these systems is challenging.

Here we use and alternative approach that relies on the study of pK_a variations of ionisable residues during molecular dynamics simulations to capture the changes in protein properties on allosteric ligand binding. This method has the advantage of taking into account a set of phenomena that are otherwise difficult to consider collectively, including backbone motions side chain fluctuations, solvent exposure and hydrogen bonding and electrostatic interactions.

To demonstrate its benefits, we used this approach to probe the allosteric communication pathway of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase from *Neisseria meningitidis*. Allosteric regulation of this enzyme is particularly challenging, as the protein does not experience major conformational changes on allosteric inhibitor binding.

P23

How electron cryotomography is opening a new window into bacterial cell biology

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In the last ten years electron cryotomography has made it possible to visualize large macromolecular assemblies inside intact cells in a near-native, "frozen-hydrated" state in 3-D to a few nanometers resolution. Increasingly, atomic models of individual proteins and smaller complexes obtained by X-ray crystallography, NMR spectroscopy, or other methods can be fit into cryotomograms to reveal how the various pieces work together inside cells. To illustrate these points, I will present a few case studies from our recent work in bacterial cell biology, including new images and mechanistic insights into bacterial chemoreceptor arrays, secretion systems, and the Type IV pilus.

P24

A NuRDs, NuDes and (re)modelling

Low, J.K.[1], Webb, S.R.[1], Silva, A.P.G.[1], Ryan, D.P.[2], Torrado, M.[1], Landsberg, M.[3], Saathoff, H.[1], Parker, B.L.[1], Shepherd, N.E.[1] and Mackay, J.P.[1]

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Chromatin acts as a roadblock to all genome biology and chromatin-remodelling complexes act to deposit, remove and rearrange these roadblocks. However, the molecular mechanisms underlying the activity of these complexes are in general not well understood.

The Nucleosome Remodeling and Deacetylase (NuRD) complex is one such epigenetic regulator. NuRD is conserved across all complex animals and is essential for normal development. Recent data also suggest that components of the complex play important roles in both the DNA damage response and age-related memory loss. Despite being described 15 years ago, almost nothing is known about the architecture of the intact complex or how it acts to alter chromatin structure.

We are using a range of biochemical and structural approaches, including single-particle electron microscopy, x-ray crystallography, NMR spectroscopy and mass spectrometry, in an effort to determine the structure and biochemical mechanism of action of NuRD. Our data provide a glimpse into the mechanisms through which complex coregulator complexes are recruited to target genes and ultimately will help to map out the molecular events that drive gene regulation.

P25

Atomic view of a generic pH-driven conformational switch motif: from pH-sensitive peptide nanotubes to biologically relevant protein conformational pH-switches

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External stimuli are powerful tools that naturally control protein assemblies and functions, whereas self-assembled nanomaterials hardly possess such responsive properties. In this context bio-inspired approaches could open the way toward tunable self-assembled architectures.

Here we report a self-assembling pH sensitive peptide that forms either highly ordered bundles of small nanotubes of 11nm diameter ($pH<6.5$) or large nanotubes of 50nm diameter ($pH>7.5$). Using a set of biophysical techniques (TEM, SAXS, IR, Raman, and crystallography), we were able to unravel the molecular mechanisms of the peptide conformational changes driving the transition between small and large nanotubes.

The sub-angström peptide crystal structure (basic pH) reveals a globular conformation stabilized through a strong histidine-serine H-bond and a tight histidine-aromatic packing. Lowering the pH affects histidine protonation, disrupts these interactions and triggers a large change to an extended beta-sheet based conformation. Re-visiting the available crystal structures (PDB) of proteins with pH dependent functions reveals both histidine-containing aromatic pockets and histidine-serine proximity as key motifs. The mechanisms unraveled in this study may thus be generally used by pH-dependent proteins and opens new prospects in the field of responsive bio-nanomaterials.

Valery et al. *Nature Communications* 2015, accepted on 9 Jun 2015, DOI: 10.1038/ncomms8771

P26

Trim5α assembles to recognise retroviral infection and activate signalling

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Vertebrate genomes show evidence of prolonged exposure to retroviral pathogens in the form of endogenous retroviral elements. Consequently, cells have developed methods to prevent and contain infection by retroviral pathogens. The post-entry restriction factor Trim5α blocks infection by a range of retroviruses, including HIV-1, in the early stage of the retroviral lifecycle, preventing the reverse transcription and integration of the virus into the host genome. Trim5α is a multi-domain protein consisting of a RING domain, with E3-ubiquitin ligase activity, a B-Box domain, a coiled-coil region and a C-terminal SPRY/PRY capsid recognition domain.

TRIM5α-mediated restriction occurs in a species specific manner and is dependent upon recognition of the lattice of capsid protein that forms the inner core of the retrovirus. As this lattice is conserved across retroviruses, TRIM5α acts as a pattern recognition receptor for the retroviral capsid activating ubiquitin based signalling events via the N-terminal RING domain[1].

The direct recognition of the capsid protein is governed by the C-terminal capsid recognition domain, however the interaction with monomeric capsid is weak[2]. Therefore, potent recognition of the capsid lattice is dependent upon the formation of dimers and higher order assemblies that generate powerful avidity effects.

To investigate assembly, capsid recognition and signalling by Trim5α restriction factors we have determined the first structure of the core self-association domain, encompassing the Bbox and coiled-coil region, by X-ray crystallography[3]. Together with data from ubiquitylation assays, we provide a model of recognition leading to activation of the E3-ubiquitin ligase activity of Trim5α and the restriction of retroviral infection.

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QMB Proteins Satellite: Summary of Posters

No.	Title	Presenter	Institutions
P27	Exploring the functional interdependency of fused enzymes	Yu Bai	University of Canterbury, New Zealand
P28	One residue to rule them all: Remote glycine required for propagation of allostery	Nicole Blackmore	University of Canterbury, New Zealand
P29	Discovery of a chemoreceptor for dipeptides in *Pseudomonas syringae* pv. *actinidiae*	Sean Boult	University of Otago, New Zealand
P30	Development of biotechnological assays based on a unique blue pigment synthesising enzyme	Alistair Brown	Victoria University, New Zealand
P31	Production of recombinant granulovirus and cypovirus polyhedra in *Escherichia coli*.	Jason Busby	University of Auckland, New Zealand
P32	Using three-dimensional structure in inferring deep homology	Toni Daly	Massey University, New Zealand
P33	Conformational Dynamics and Allostery in Pyruvate Kinase	Katherine Donovan	University of Canterbury, New Zealand
P34	Characterisation of Itch: A ubiquitin-ligase and protein-protein interaction hub	Michael Fairhurst	University of Otago, New Zealand
P35	Crystallographic analysis of an unusual posttranslational modification, a cysteine-tyrosine crosslink, in thiol dioxygenases	Matthias Fellner	University of Otago, New Zealand
P36	Exploring the role of a dynamic accessory domain	Fiona Given	University of Canterbury, New Zealand
P37	Investigating primordial enzyme evolution with *Thermotoga maritima* CBL: A modern example of a multitasking enzyme	Kelsi Hall	University of Otago, New Zealand
P38	Peptide based medicine – the next step in medicine	Thomas Handley	University of Otago, New Zealand
P39	Uncovering hidden allostery	Logan Heyes	University of Canterbury, New Zealand
P40	Computational investigations of allosteric regulation mediated by a molecular pendulum	Wanting Jiao	University of Canterbury, New Zealand

P41	Structural insights into the catalytic cycle and conformational changes of SEPHCHC Synthase (MenD), an essential ThDP-dependent enzyme from the menaquinone biosynthesis pathway in *Mycobacterium tuberculosis*	Jodie Johnston	University of Auckland, New Zealand
P42	Characterisation of assembly of the antiretroviral protein Trim5α	Jeremy Keown	University of Auckland, New Zealand
P43	The dynamics of change: the adaptive evolution of an allosterically-regulated enzyme in *E. coli*	Sarah Kessans	University of Canterbury, New Zealand
P44	Engineering cytochrome $P450_{BM3}$ as biocatalysts for steroid compounds	Sam Kim	[1]Callaghan Innovation, [2]University of Auckland New Zealand
P45	Nuclear factor kappa B (NF-κB) proteosomal degradation by C-terminus of Hsc70-interacting protein (CHIP) protects cardiomyocytes from advanced glycation end- products (AGEs)- induced apoptosis	Wei-Wen Kuo	China Medical University
P46	High-density lipoprotein (HDL) reverses palmitic acid-induced energy metabolism imbalance by switching CD36 and GLUT4 signaling pathways in cardiomyocyte	Chih-Yang Huang	[1]China Medical University [2]Asia University
P47	Characterization of methanogen nonribosomal peptide assembly lines	Anders Laegaard	University of Auckland, New Zealand
P48	Does $HisG_S$ really need it's friend?	Emma Livingstone	University of Canterbury, New Zealand
P49	Cofactor engineering of a clostridial alcohol dehydrogenase leads to unexpected changes in substrate specificity	Danielle Maddock	University of Otago, New Zealand
P50	Understanding how *Phytophthora* 'sniff out' their hosts	Alison McGhie	University of Otago, New Zealand
P51	A high-throughput screen for ligand binding reveals three amino acid chemoreceptors from *Pseudomonas syringae* pv. *actinidiae*	James McKellar	University of Otago, New Zealand
P52	A plant-like PPR protein controls RNA cleavage in the *Plasmodium* remnant chloroplast	Joanna McKenzie	Cambridge University, UK
P53	Long vs Short; Transition state analysis of adenosine triphosphate phosphoribosyl transferases.	Gert-Jan Moggre	University of Canterbury, New Zealand

P54	Engineering biofilm-blocking enzymes	Shereen Murugayah	University of Otago, New Zealand
P55	Investigating the peroxidase reaction mechanism of human cytochrome *c*.	Rinky Parakra	University of Otago, New Zealand
P56	Pinpointing the divergence of quaternary structure in the lysine biosynthetic pathway	Grant Pearce	University of Canterbury, New Zealand
P57	Biophysical characterisation of chemotaxis receptor-ligand interactions from the Kiwifruit bacterial pathogen *Pseudomonas syringae* pv. *actinidiae*	Jodi Pilbrow	University of Otago, New Zealand
P58	Engineering an optimised selection system for folded proteins	Gabriel Rawcliffe	University of Otago, New Zealand
P59	Structural vaccinology of Group A Streptococcus Pili	Jeremy Raynes	University of Auckland, New Zealand
P60	Expression and purification of Keratin Associated Proteins for structural and biophysical characterization	Othman Rechiche	University of Auckland, New Zealand
P61	Development of positive selection methods to improve nitroreductase activity by directed evolution	Michelle Rich	Victoria University, New Zealand
P62	Directed Evolution of Bacterial Enzymes to Create Powerful Tools for Cellular Ablation Studies	Abby Sharrock	Victoria University, New Zealand
P63	Reconstruction of Ancestral Enzymes from a Core Biosynthetic Pathway	Konny Shim	University of Waikato, New Zealand
P64	Design and optimisation of a screen to enable directed evolution of superior DNA ligases	Luke Stevenson	Victoria University, New Zealand
P65	The structure of a rumen bacteria glycoside hydrolase 29 family αLfucosidase reveals unique dual carbohydrate binding domains	Emma Summers	University of Waikato, New Zealand
P66	Structural Characterisation of Menangle virus Nucleocapsid-like Particles	Melissa Webby	University of Auckland, New Zealand
P67	Investigation of a putative proton relay chain in the enzyme 3-deoxy-D-manno-octulosonate 8-phosphate synthase	Michael Weusten	University of Canterbury, New Zealand
P68	Engineering quorum quenching enzymes to prevent *Pseudomonas aeruginosa* biofilm formation	Thomas Wiggins	University of Otago, New Zealand

P69	The structure of Cas1 and its role in adaptation in Type I-F CRISPR-Cas systems	Max Wilkinson	University of Otago, New Zealand
P70	Evolving bacterial nitroreductase enzymes for improved activation of a challenging next-generation prodrug substrate	Elsie Williams	Victoria University, New Zealand
P71	Snakin-1: a racemic success story	Ho Yeung	University of Auckland, New Zealand
P72	Nano-LEGO: Making it CLICK!	N. Amy Yewdall	University of Canterbury, New Zealand
P73	Characterisation of RNF11 – a negative regulator of immune signalling	Jingyi (Gene) Zhu	University of Otago, New Zealand
P74	Towards understanding the role of non-ribosomal peptide synthetases in methanogenic archaea	Verne Lee	University of Auckland, New Zealand

Poster Abstracts P27-P72

P27

Exploring the functional interdependency of fused enzymes

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My project concentrates on the 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase (DAH7PS) from *Prevotella nigrescens* (*Pni*DAH7PS), a DAH7PS with a C-terminal chorismate mutase (CM) domain. DAH7PS is the first enzyme of the shikimate pathway, which is responsible for the production of aromatic metabolites inmicroorganisms and plants. Via six subsequent reactions, DAH7P is converted to chorismate. CM is another pivotal enzyme in shikimate pathway, converting chorismate to prephenate, which leads to the aromatic amino acids Phe and Tyr. Both DAH7PS and CM are the major control points for the regulation of the rate of production of the aromatic amino acids. The core structure of DAH7PS that provides a basic catalytic activity is a $(\beta/\alpha)_8$ barrel. The core catalytic barrels have been found to be decorated with various insertions or extensions in most of the known bacterial DAH7PSs, and these extra elements are generally considered to provide the structural machinery for the allosteric regulation of DAH7PS.

However my research strongly suggests that the function of the C-terminal extension, a CM domain, of *Pni*DAH7PS may be more important than simply providing for allosteric regulation. The kinetic characterisation of the catalytic activities of the separated DAH7PS and CM domains of *Pni*DAH7PS revealed that the both CM and DAH7PS activities were considerably attenuated by their separation, indicating a functional dependency between the two domains may exist. A reasonable explanation for this observation is available from the rigid body model for ligand-free *Pni*DAH7PS generated from small angle X-ray scattering (SAXS) data, which predicts that interactions exist between the active sites of DAH7PS and CM domains, and that these interactions may significantly support both the catalytic activities of DAH7PS and CM. Moreover, further analysis of SAXS data and the model for prephenate-bound *Pni*DAH7PS indicates that these interactions may be broken by prephenate binding, meaning that CM and DAH7PS domains no longer benefit from their fusion, providing a model for the allosteric regulation of this protein. If this proposed model speculation is verified by future work, it would present a distinctive regulatory mechanism of DAH7PS activity, and also would provide a new viewpoint about the function of gene fusion in the evolution of DAH7PS.

P28

One residue to rule them all: Remote glycine required for propagation of allostery

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The DAH7PS from *Mycobacterium tuberculosis* (*Mtu*DAH7PS) is synergistically feedback regulated by binary and ternary combinations of the aromatic amino acids Trp, Phe and Tyr, without undergoing a noticeable conformational change.[1] Furthermore, *Mtu*DAH7PS is responsible for the feedback regulation of a second enzyme, chorismate mutase from the same organism, on formation of a non-covalent hetero-octameric complex.[2,3]

Molecular dynamic simulations conducted on *Mtu*DAH7PS highlight changes in flexibility that occur on ligand binding.[4] Correlation of the molecular dynamics simulations with a statistical coupling analysis identified Gly190, located at the start of a flexible loop, as a potentially critical residue. This loop is, located at the subunit interface of the homo-tetramer and is remote from the allosteric binding sites and the active site. The role of Gly190 has been probed via site directed mutagenesis.

Presented here are the findings of the mutagenesis study where Gly190 was mutated to a proline, introducing conformational constraints on the local backbone flexibility at the start of the flexible loop. These results show how the Gly190Pro mutation disrupted the allosteric regulation of *Mtu*DAH7PS, altered the enzyme's ability to regulate its non-covalent binding partner *Mtu*CM and more curiously the effect the mutation has had on the enzyme's quaternary structure and function.

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P29

Discovery of a chemoreceptor for dipeptides in *Pseudomonas syringae* pv. *actinidiae*

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The perception and processing of chemical signals (*e.g.* food, toxins) from the environment is essential for any living organism. In bacteria, motility specific responses can be achieved via the coordinated sensory input of methyl-accepting chemotactic proteins (MCPs). Compared to enteric bacteria, the chemotactic behaviour of environmental microorganisms is poorly understood. This comes despite the fact genome analyses have revealed that soil bacteria generally encode a larger number of MCPs. The sequenced genome of *Pseudomonas syringae* pv*. actinidiae* (*Psa*) strain NZ-V13 is predicted to encode 43 putative MCPs. Recently, our group has identified three *Psa* chemoreceptors that specifically detect D- and L-amino acids. However, with regard to the remaining MCPs, neither what they detect nor how they detect it is understood.

To address these questions, three additional putative *Psa* MCPs (locus IDs Psa_00575, Psa_25325 and Psa_26253) have been selected for investigation. Using a high-throughput fluorescence based thermal shift assay, the ligand binding domains (LBDs) of each MCP have been screened against ~500 different potential ligands. With Psa_00575 found to bind dipeptides, progress towards the biophysical and structural characterisation of this LBD will be presented. Understanding the structural basis of molecular recognition has implications for the fields of biochemistry and synthetic biology attempting to develop protein based technologies. However, whether or not this reflects a major physiological significance for *Psa* remains to be determined. Screening of the remaining MCPs will be completed in the near future.

P30

Development of biotechnological assays based on a unique blue pigment synthesising enzyme.

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Blue pigment synthase A (BpsA) is a single module Non-Ribosomal Peptide Synthetase (NRPS) originally isolated from the bacterium *Streptomyces lavendulae.* It synthesises an easily detectible blue pigment called indigoidine from two molecules of L-glutamine in an ATP powered reaction.

By spectrophotometrically quantifying indigoidine levels it is possible to accurately measure the amount of L-glutamine in complex biological fluids including urine, blood plasma and cell culture media. This method has several advantages over existing commercial kits for glutamine measurement. This includes the fact that it directly reports on glutamine levels. All other commercially available kits convert glutamine into glutamate and then measure the level of glutamate, which requires additional sample processing and introduces complexity if glutamate is also present in the target solution.

A BpsA based assay to detect 4'-phosphopantetheinyl transferase (PPTase) inhibitors has also been developed. PPTases are essential enzymes that attach a phosphopantetheine arm to fatty acid synthases, NRPSs and polyketide synthases. This attachment switches the enzymes from an inactive *apo*-form to an active *holo*-form. PPTases have been validated as promising drug targets, but there have not previously been suitable assays to screen directly for inhibitors of PPTases from pathogenic bacteria. In order to detect PPTase inhibitors, we have shown that BpsA can be purified in its inactive *apo* form and mixed with the target PPTase as well as a candidate inhibitor *in vitro*. The level of PPTase inhibition can then be calculated by measuring the amount of indigoidine produced. The assay has been optimised for high throughput screening and used to identify several compounds from chemical libraries that inhibit essential PPTases of *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis.*

P31

Production of recombinant granulovirus and cypovirus polyhedra in *Escherichia coli*

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Polyhedra are stable protein microcrystals produced inside insect cells after infection with baculoviruses or cypoviruses. These microcrystals encapsulate and protect the virus particles, allowing them to resist environmental degradation. Cypoviruses are members of the *Reoviridae* family of double-stranded RNA viruses that produce polyhedra in the cytoplasm of infected cells and baculoviruses are members of *Baculoviridae*, double-stranded DNA viruses that produce polyhedra in the nucleus.

Polyhedra are remarkably stable, able to resist treatments that would denature most proteins including 8 M urea, detergents, dehydration, alcohols, and acids[1]. They dissolve readily, however, at the high pH of the insect mid-gut.

The high stability, conditional dissolution, and amenability to protein engineering makes viral polyhedra promising candidates for the engineering of nanodevices and nanomaterials. The structures of baculovirus and cypovirus polyhedra have been determined[1,2]. CPV polyhedra have nucleotides incorporated into the crystal lattice[1], suggesting that it might be possible to engineer them to contain other small molecules. These polyhedra can also be engineered to incorporate foreign proteins by co-expression with an inclusion-tagged protein[3].

Previous attempts to engineer polyhedra were performed in insect cell culture. This approach has several drawbacks, including the high cost of cell culture media, low total yield, and long clone-to-protein turnaround time. We have produced silkworm (*Bombyx mori*) cypovirus and codling moth (*Cydia pomonella*) granulovirus polyhedra in *Escherichia coli*. This system allows the rapid production and characterisation of modified polyhedra via standard molecular biology techniques, and cheap large-scale production. We show that bacterially expressed polyhedra are crystalline and produce the same powder diffraction pattern as wild-type polyhedra. We also show the incorporation of GFP into bacterially produced polyhedra in a similar manner to insect cell expression.

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P32

Using three-dimensional structure in inferring deep homology

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Typically researchers have used primary sequence homology of DNA and protein to build trees and infer relationships. This approach as at least two limitations. Firstly, at the deepest levels of phylogeny the Markov models used are expected to saturate, and lose information, and our confidence of the relationships at these levels is lost. Secondly, we need to understand much more about the nature and principles of living systems. In many cases substitutions, even deletions and insertions may not strongly affect the ultimate structure at the tertiary and quaternary level. We can anticipate that structural homology is likely to be retained long after sequence homology has all but evolved away.

In our studies we have used freely available servers to predict the structure of extant proteins, as well as putative ancestral sequences. We then use these to either BLAST to find more sequences or to infer ancestral 3D structure. We can check that catalytic sites are retained with a geometry that suggests that they would retain function and we have also used docking algorithms to check the likelihood of spontaneous assembly. Our targets to date have been the Major Vault Protein (MVP) that spontaneously form a vault particle and the Argonaute/PIWI proteins whose expansion is in some cases astonishing (and puzzling) and whose known functional capabilities are expanding with every publication.

We have found a simple and inexpensive method of looking farther into the past to try and resolve those puzzles not amenable by BLASTing alone. From this approach, protein sequences can be screened and ruled in or out before expensive lab work is undertaken.

P33

Conformational Dynamics and Allostery in Pyruvate Kinase

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To elucidate the conformational and dynamic changes that drive heterotopic allosteric activation of Escherichia coli pyruvate kinase type 1 by fructose-1,6-bisphosphate, we performed a time-resolved electrospray ionization mass spectrometry coupled to hydrogen-deuterium exchange study. The global exchange experiment demonstrates that fructose-1,6-bisphosphate binding to the allosteric domain of pyruvate kinase causes a shift towards a globally more unstructured and dynamic ensemble of conformations. Mapping deuterium exchange to peptides within the protein highlights site-specific regions with altered conformational dynamics upon fructose-1,6-bisphosphate binding, many of which increase conformational flexibility. We propose an allosteric mechanism whereby the binding of the activator, fructose-1,6-bisphosphate, destabilises an ⍰-helix that bridges the allosteric and active site domains within the monomeric unit. This destabilises ⍰-strands within the active site (⍰⍰⍰)8-barrel domain that are linked to the active site loops, where increased flexibility of substrate binding loops increases substrate binding affinity. Lastly, the tetrameric interfaces are destabilised, explaining why the protein no longer shows cooperativity with respect to phosphoenolpyruvate when fructose-1,6-bisphosphate is bound, since the interfaces of the monomers within the tetramer are now decoupled. This work extends previous knowledge of the allosteric activation mechanism by explaining how fructose-1,6-bisphosphate promotes substrate binding, despite the allosteric binding site being 40 Å away from the active site.

P34

Characterisation of Itch: A ubiquitin-ligase and protein-protein interaction hub

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The E3 ubiquitin-protein ligase Itchy homologue (Itch) is an important negative regulator of inflammatory signalling and the viral immune response, while also controlling aspects of cellular differentiation and receptor sorting to lysosomes[1]. Itch regulates these processes through ubiquitylation of cellular proteins catalysed by the homologous to E6-AP carboxy terminus (HECT) domain. In addition to the HECT domain, Itch has four WW domains that bind to proline-rich motifs. This interaction facilitates complex formation with other enzymes or adapters and directly recruits substrates for ubiquitylation. However, little is known about how multiple WW domains function in tandem. Furthermore, an internal proline-rich region (PRR) has been reported to inhibit the activity of Itch through unknown mechanisms[2].

Using pull-down assays and biolayer interferometry, the interaction between the WW domains and RNF11, a model substrate, have been examined. As expected, the WW domains of Itch strongly bind to the canonical PPxY motif found in RNF11. However, the WW domains also bind to two other non-canonical PxPx motifs. The effect of the PRR is also being investigated. The PRR did not seem to exert significant effect on substrate recruitment by the WW domains. However, using in vitro ubiquitylation assays, we observed reduced RNF11 ubiquitylation in the presence of the PRR.

In summary, we have shown that RNF11 displayed specific and avid interactions with the WW domains of Itch. Furthermore, despite the fact that RNF11 ubiquitylation was mildly impaired in the presence of PRR, the substrate interaction was not inhibited, contrary to our initial hypothesis. Future studies are required to strenghten these findings, and will undoubtedly shed light on the mechanism of Itch-mediated ubiquitylation.

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P35

Crystallographic analysis of an unusual posttranslational modification, a cysteine-tyrosine crosslink, in thiol dioxygenases

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The thiol dioxygenase cysteine dioxygenase (CDO) oxidises cysteine to its sulfinic acid, the first step in oxidative breakdown of cysteine in mammals. Due to its role in regulating cysteine levels it is linked to various diseases including cancer, rheumatoid arthritis and several neurological diseases, such as Alzheimer's and Parkinson's diseases. Crystal structures of mammalian CDO show a ferrous ion in the active site coordinated by three histidines and in close proximity to an unusual posttranslational modification, a covalent cysteine-tyrosine crosslink between C93 and Y157. Although the cysteine and tyrosine which form the crosslink are completely conserved in eukaryotes, sequence alignment of putative CDOs in prokaryotes shows that only the tyrosine is conserved and the position equivalent to C93 in mammals is often replaced by a glycine.

Here we present a crystallographic investigation of this crosslink and other features of this enzyme family by comparing the eukaryotic CDO from Rattus norvegicus and a prokaryotic, uncrosslinked CDO from Pseudomonas aeruginosa. Through the use of different crystal growth conditions, site directed mutagenesis and ligand soaking, we were able to obtain several new crystal structures with distinct yet unseen features at the active site. Ligands bound in the active site give further insight into the possible mechanism of the CDO reaction. Comparing the active site pocket residues in the mammalian and prokaryotic CDO structures offers insight into the roles of residues involved in the crosslink and other residues believed to be important for catalysis, contributing to the understanding of the dioxygenation of thiols by this family of enzymes.

P36

Exploring the role of a dynamic accessory domain

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Isopropylmalate synthase (IPMS) catalyses the first reaction in the leucine biosynthetic pathway in bacteria, fungi, and archaea. This enzyme is allosterically regulated by leucine. IPMS is a homodimer and each chain consists of a catalytic domain, two subdomains which act as a catalytic accessory unit, and a regulatory domain1. The binding site for leucine is located over 50 Å away from the active site of the enzyme. The mechanism of allosteric regulation is not fully understood. IPMS is closely related to homocitrate synthase (HCS), which functions in the lysine-producing α-aminoadipate pathway that is present in fungi and some archaea and bacteria2. HCS is structurally very similar to IPMS but lacks a regulatory domain and instead is competitively inhibited by lysine.

In our studies of the IPMS from Neisseria meningitidis, we have demonstrated that a functional, leucine-insensitive, enzyme can be created by removal of the regulatory domain as long as subdomain II of the catalytic accessory unit remains intact. This finding has allowed us to explore the roles of this catalytic accessory unit in both catalysis and leucine regulation.

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P37

Investigating primordial enzyme evolution with *Thermotoga maritima* CBL: A modern example of a multitasking enzyme.

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It has been proposed that the earliest enzymes each possessed a range of activities. This differs from current day enzymes, which typically have single and specific functions. The cystathionine β-lyase (CBL) from *Thermotoga maritima* is unusual as it can conduct three essential yet distinct reactions, similar to the ways that primordial enzymes may have acted. In addition to cystathionine elimination, *T. maritima* CBL is also able to catalyse the racemisation of alanine and glutamate, albeit poorly.

One difference between the specialist *E. coli* CBL and the multi-functional *T. maritima* CBL is a tryptophan residue (W108) in the active site of the *T. maritima* enzyme. This residue was subjected to site-directed mutagenesis, to assess the role it plays in the three different reactions. One of the mutations introduced, W108F, resulted in an improvement in alanine racemisation, while still retaining cystathionine elimination and glutamate racemisation activities. Error prone PCR was also used in an attempt to improve one or more of these activities. This information is providing insights into how ancient enzymes with broad specificities may have evolved to possess single and specific functions.

There are no known alanine racemase homologues in the *T. maritima* genome. While CBL can catalyse alanine racemisation, it remained unknown whether it was the physiologically relevant alanine racemase. A screen of a *T. maritima* open reading frame (ORF) library was conducted, to search for alanine racemase candidates other than CBL. This screen has tentatively identified a new alanine racemase candidate, unlike anything previously identified. Initial results of our efforts to characterise it will be presented.

P38

Peptide based medicine - the next step in medicine.

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Modern medicine is reliant on small molecules as therapeutics’. Naturally occurring and rationally designed small molecules have limited specificity as a result of their small size. Recently, peptides have been investigated as a potential complement to small molecule medicines; the increase in size permits an increase in target specificity and discrimination[1]. Currently the FDA has approved more than 60 peptide drugs for public use with an additional 500+ in development and trials.

Peptide therapeutics can come from many different natural sources, commonly venoms and plants. Linear peptides often have limited application as therapeutic compounds because they are rapidly degraded[2]. Cyclotides (peptides with their N- and C- termini joined by a peptide bond) offer an ideal therapeutic alternative to linear peptides, as they are capable of resisting both chemical and physical degradation[3]. The production of cyclic variants of natively linear peptides is an appealing strategy for enhancing the *in vivo* stability of pharmaceutically appealing peptides, dramatically improving their therapeutic capacity.

There is a range of strategies available for the generation of cyclotides, which fall into two classes: chemical or biosynthesis. The complicated nature of chemical synthesis puts an exclusive price tag on chemically synthesized cyclic peptides, prohibiting wide scale application in medicine[4]. Conversely, biosynthesis approaches require large amounts of initial optimization, with minimal subsequent expenses. Which makes the system ideal for the production of affordable large scale pharmaceutical compounds. The most appealing biosynthesis approach is the SICLOPPs (Split Intein mediated CycLisation Of Proteins and Peptides)[5]. SICLOPPs takes advantage of the indiscriminant peptide ligation capacity of split inteins to ligate the N- and C- termini of the target peptide together through a native peptide bond to facilitate cyclisation of the target peptide. To facilitate cyclisation the split intein subunits are cloned onto the termini of the target peptide for expression in a bacterial system, following translation into the full length protein the split intein subunits activate spontaneously and peptide cyclisation occurs.

With the SICLOPPs system we see a lot of undesirable protein cyclisation within the cell, limiting the final yield of the system (often as low as a 2% final yield). To overcome this unacceptable loss, we are exploring the possibility of developing conditional split inteins for the SICLOPPs system.

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P39

Uncovering hidden allostery

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Allostery is the process by which the binding of a ligand to a protein induces a functional response at a remote site, through an alteration in structure and/or dynamics. The enzyme 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase (DAH7PS) catalyses the first committed step of the shikimate pathway, responsible for the biosynthesis of important aromatic compounds, including the amino acids phenylalanine, tyrosine and tryptophan.[1] The flux of this pathway is controlled at the protein level via allosteric regulation. *Neisseria meningitidis* DAH7PS is most potently allosterically regulated by phenylalanine.

There is no significant structural change associated with phenylalanine binding to *Nme*DAH7PS, implying that this enzyme relies on subtle changes in dynamics to achieve appropriate regulation. Molecular dynamics simulations were employed to calculate pKa variations in ionisable residues associated with phenylalanine binding. The findings implicate several residues that are involved in a hydrogen bonding network, hypothetically responsible for communication of the allosteric signal between remote sites. Several point variants have subsequently been made to test this hypothesis. The results of which illustrate the importance of the dynamic properties of *N. meningitidis* DAH7PS for allostery and enable the signal transduction pathway between allosteric and active sites to be delineated.

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P40

Computational investigations of allosteric regulation mediated by a molecular pendulum

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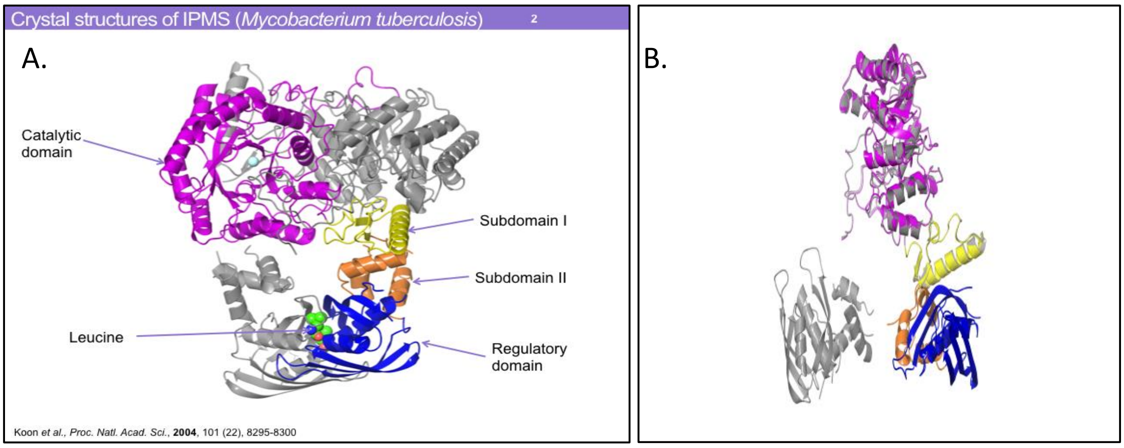
The enzyme α-isopropyl malate synthase (α-IPMS) catalyzes the first committed step of the biosynthesis of the amino acid leucine, and is feedback regulated by the binding of leucine to distant allosteric sites. α-IPMS from *Mycobacterium tuberculosis* is a homodimer, and each chain consists of a catalytic core with regulatory domain attached *via* an accessory domain, which can be further classified into two subdomains (Fig 1A). The asymmetry comes from the different positions of subdomain I and II of the accessory domain relative to the catalytic core, and gives rises to two different conformational extremes for each chain (Fig 1B). It is possible then each chain can oscillate between the two conformations (as a molecular pendulum) and this oscillation may be critical for catalysis.



Koon *et al.*, *Proc. Natl. Acad. Sci.*, **2004**, 101 (22), 8295-8300

Figure 1 (A) Crystal structure of α-IPMS from *Mycobacterium tuberculosis*. (B) Asymmetry in monomer conformations.

Our hypothesis is that the catalysis and allosteric control of α-IPMS are mediated by this molecular pendulum motion. Specifically, α-IPMS oscillates between the two asymmetric conformational extremes, and leucine binding disrupts enzymic function by altering the dynamics (both the amplitude and frequency) of this swinging pendulum assembly. Computational studies for both α-IPMS and other related enzymes were conducted to investigate the dynamics of the accessory domains and their role in driving the pendulum motion and hence catalysis.

P41

Structural insights into the catalytic cycle and conformational changes of SEPHCHC Synthase (MenD), an essential ThDP-dependent enzyme from the menaquinone biosynthesis pathway in Mycobacterium tuberculosis

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Menaquinone (MQ) is an essential component of the respiratory chains of many pathogenic organisms, including Mycobacterium tuberculosis (Mtb). In Mtb, MQ is needed even in the persistent state and there is interest in targeting MQ biosynthesis enzymes as drug targets. The first committed step in MQ biosynthesis is catalyzed by MenD (2-succinyl-5-enolpyruvyl-6- hydroxy-3-cyclohexadiene-1-carboxylate synthase), a thiamine diphosphate (ThDP)-dependent enzyme. The ThDP co-factor, activated by enzyme binding, plays a core role in catalysis, which proceeds through two covalent ThDP-bound intermediates; the first after 2oxoglutarate binding and decarboxylation, the second upon ligation of isochorismate to the first intermediate. Cleavage of the product then follows.

We have expressed Mtb MenD, confirmed activity via NMR and UV spectroscopy and determined a series of crystal structures; ligand-free, with co-factor alone, and with substrate and covalent intermediates bound. This has enabled us to map the binding sites of both substrates and visualize the steps in the MenD catalytic cycle. Mtb MenD is a tetrameric enzyme, a dimer of dimers with each dimer active site comprising contributions from both monomers. We observe a marked asymmetry upon ThDP binding, with only one active site per dimer occupied. This suggests Mtb MenD, like some other ThDP-dependent enzymes, exhibits “half-of-sites” reactivity, and that the two active sites in the dimer communicate with each other. There is a hydrogen bonding network between both active sites in the dimer, and a set of key mobile regions in the active site that change conformation upon ThDP binding. This suggests plausible mechanisms for communication between the paired active sites. The crystal structures also identify features of the two intermediates that facilitate reaction but prevent premature product release, and identify a role for a key glutamine residue in the active site. These data provides useful information to inform early-stage drug discovery.

P42

Characterisation of assembly of the antiretroviral protein Trim5α

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The post-entry restriction factor Trim5α blocks retroviral infection by preventing reverse transcription and integration into the host genome. As a member of the Trim protein family Trim5α shares a conserved N-terminal domain architecture comprising a RING, B-box, and coiled-coil domain. The C-terminal PRY/SPRY domain determines the range of retroviruses Trim5α recognizes through direct interaction with the retroviral capsid protein. However, the affinity of this interaction is weak.[1] Consequently, multiple binding events are required to activate restriction. To achieve this Trim5α has been shown to assemble into a hexagonal array[2] which is complementary to the retroviral capsid lattice, greatly increasing the strength of the interaction with the retroviral capsid.

To investigate the nature of this assembly we have undertaken a structural and biophysical characterization of Trim5α from Rhesus macaques. Previously we have determined the structure of the B-box and coiled-coil domains and shown them to assemble as an elongated antiparallel dimer, placing the B-box domain at opposite ends of the central coiled-coil dimer.[3] The length of the coiled-coil is consistent with forming an edge of the hexagonal array, acting as a scaffold. This places both the B-box and RING domains at the vertices, forming a three-fold interface.

To characterize this three-fold interface we have designed a Trim5α construct comprising the B-box and a truncated coiled coil domain that is incapable of forming the complete dimer. Using sedimentation velocity and sedimentation equilibrium analytical ultracentrifugation we have characterized the strength and nature of the B-box assembly. Using small-angle X-ray scattering we have determined low resolution molecular envelopes for monomeric and assembled oligomers. The docking of high-resolution structures has provided a model describing the complete formation of the Trim5α hexagonal assembly.

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P43

The dynamics of change: the adaptive evolution of an allosterically-regulated enzyme in *E. coli*

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Although we have a good understanding of adaptation at the organismal level, there is a paucity of data addressing how organisms adapt at the molecular level. We ask how enzymes evolve: what is evolution trying to optimise? Our study builds upon a laboratory experiment in which twelve replicate bacterial populations were evolved from a common ancestor in a glucose-limited environment. The fitness of each evolved population increased relative to the ancestor over successive generations, indicating adaptation to the experimental environment. Whole genome and candidate gene sequencing found that one gene, pykF, encoding the regulatory, glycolytic enzyme, pyruvate kinase, was mutated in all twelve populations. Our study aimed to understand why pykF was a focal point for mutations. To examine whether the evolved pykF alleles conferred a fitness benefit in and of themselves, we replaced the wild-type pykF gene in the ancestor with each of the evolved pykF genes. Competitive fitness assays demonstrated that although each of the pykF genes conferred a fitness benefit, the magnitude of the fitness benefit was different in each case. We found the structures of the mutants nearly identical to that of the ancestral enzyme, while the functions of the mutants in respect to activity, substrate binding and allosteric regulation, varied dramatically. Comparing B-factor profiles of our structures hinted at differences in protein dynamics, so we turned to hydrogen/deuterium exchange experiments, which demonstrated that the evolved enzymes are dynamically different. Results of metabolomic analysis suggest that the altered functions of the enzymes lead to a shift in metabolomic flux during growth on low glucose. In conclusion, our results point to protein dynamics as an important mode for adaptive evolution in proteins: in this case, evolution seems to be acting on protein dynamics to optimise enzyme allosteric function in order to help organisms adapt to environmental changes.

P44

Engineering cytochrome $P450_{BM3}$ as biocatalysts for steroid compounds

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Steroid hormones are chemical messengers that play crucial roles in both physiological and pathological processes. Steroidogenic enzymes and steroidal receptors are the prime therapeutic targets for numerous high-profile diseases, and there are continuous demands to improve, modulate, or inhibit the biological effects of these targets through steroid derivatives. One of the key limitations in the synthesis of steroid derivatives is the reliance of pre-existing functional groups in the parental steroid compounds to perform chemical transformations. If additional functional groups can be incorporated into the parental steroid compounds, then the chemical diversity of the derivatives can be greatly expanded. Unfortunately, functionalising unactivated C-H bonds remains a challenging task in organic chemistry resulting in low yields and lack of stereospecificity and regiospecificity. There is a pressing need for a more efficient method of functionalising steroid starting materials.

In this project, we aim to engineer cytochrome $P450_{BM3}$ as a biocatalyst for functionalising steroid compounds. Cytochrome $P450_{BM3}$ is a monooxygenase isolated from *Bacillus megaterium* which has been proven to be a versatile and highly evolvable enzyme. Based on published literature and crystal structures, we have created a number of cytochrome $P450_{BM3}$ mutants, and assayed them against a panel of steroid substrates. Preliminary results show that the cytochrome $P450_{BM3}$ mutants could hydroxylate numerous steroids including androgens, oestrogens and progestins. We are examining the crystal structures of the mutant enzymes to understand the basis of mutations that are beneficial or harmful to cytochrome $P450_{BM3}$ to accept steroid compounds as substrates.

P45

Nuclear factor kappa B (NF-κB) proteosomal degradation by C- terminus of Hsc70-interacting protein (CHIP) protects cardiomyocytes from advanced glycation end- products (AGEs)- induced apoptosis

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Background: Previous study showed that glycation end- products (AGEs) will bind to its receptor, RAGE, and activate apoptosis pathway via ROS generation and endoplasmic reticulum (ER) stress. C-terminus of Hsc70-interacting protein (CHIP), a co-chaperone of HSP70/90 and E3 ubiquitin ligase, can regulate protein folding and degradation. It has been identified that CHIP can reduce the damages caused by AGEs via targeting the downstream of ROS and ER stress and acts as a protector in cardiomyocytes, but the mechanism is unclear.

Results: Our data reveals that cardiac expression of CHIP is lower than normal group in diabetic and high fat diet-fed animals. In addition, we found that AGE can induce cardiac apoptosis, hypertrophy, ER stress, ROS generation, and reduce survival pathway activity, which were rescued following the overexpression of CHIP in H9c2 cells. For the examination of protein levels of NF-κB, a transcription factor which can induce ER stress, it was observed that comparing with control group, the stability of NF-κB is stronger when we knocked down, but not overexpressed, CHIP. Additionally, the IP analysis results showed that CHIP overexpression can reduce NF-κB accumulation, as well as enhanced the ubiquitination levels, which were increased following the treatment of MG132, indicating NF-κB proteosomal degradation by CHIP. These findings were agreed with the evidence that CHIP overexpression reduced the levels of NF-κB from nuclear fraction and translocation examined by western blot and immunofluorescence assay, respectively. Furthermore, U-box domain mutation abolished the reduced accumulation induced by CHIP overexpression, indicating the U-box domain involved in the association with NF-κB for its degradation.

Conclusion: These findings reveal that CHIP might act as an E3 ubiquitin ligase of NF-κB to promote its proeosomal degradation for protecting cardiomyocytes from the damages, including ER stress and ROS mediated apoptosis following AGE exposure.

P46

High-density lipoprotein (HDL) reverses palmitic acid-induced energy metabolism imbalance by switching CD36 and GLUT4 signaling pathways in cardiomyocyte

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Background: Metabolic syndromes increase the risk of developing cardiovascular disease. The risk factors for development of Metabolic Syndrome are elevated blood pressure, High fasting blood sugar, high serum triglycerides, low high-density lipoprotein (HDL) levels, and obesity. Acute toxicity from accumulation of long-chain fatty acids may lead to cell dysfunction and results in heart failure and diabetic cardiomyopathy, a phenomenon known as lipotoxicity. The occurrence of lipotoxicity induces energy imbalance. Cardiomyocytes use fatty acid as well as glucose for energy production. These substrates are transported into the cell by fatty acid transporter CD36 and the glucose transporter 4 (GLUT4). These two receptors have switching phenomenon, in this work we focus on how palmitic acid induces traffic and switch between these two receptors. On the other hand, HDL was evidenced that therapies intended to reduce the risk of cardiovascular disease. Recent study demonstrated that HDL related with energy flux. In this study, we investigated the HDL reverse energy metabolism imbalance by switching CD36 and GLUT4 signaling pathway.

Materials and Methods: To understand how palmitic acid induces energy imbalance, switching of CD36 to GLUT4. H9c2 cardiacmyoblast cells were treated with palmitic acid for 24hr. The western blotting and ELISA were performed and the results were interpreted.

Conclusion: From the western blot and ELISA, we found palmitic acid induced the imbalance in energy metabolism and the switching of CD36 to GLUT4. HDL reverses palmatic acid-induced energy metabolism imbalance in H9c2 cardiomyoblast cells and Neonatal Rat Cardiomyocyte.

Key Words: Palmitic acid; obesity; energy metabolism; CD36; GLUT4; HDL.

Abbreviation: CD36, cluster of differentiation 36; GLUT4, glucose transporter type 4; HDL, high-density lipoprotein.

P47

Characterization of methanogen nonribosomal peptide assembly lines

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Recently two NRPS genes were identified in each of the genomes of the methanogenic archaea, *Methanobrevibacter* sp. SM9 and *Methanobrevibacter ruminantium* M1[1], representing the first archaeal NRPS genes to be identified. Though the distinct modular makeup of the NRPSs were determined through *in silico* modeling no close homologues could be identified in non-redundant sequence databases, highlighting the unique makeup of the archaeal NRPS genes. The closest NRPS homolog is encoded by the bacteria, *Syntrophomonas wolfei* (isolated from anaerobic digester sludge) having an amino acid sequence identity of only 32% (PSI-BLAST). Several other NRPS genes identified in these database searches were from organisms living in the rumen or similar environments, possibly revealing a common biological theme, however no functional data is available and the non-ribosomal peptides that they syntesise have not been identified.

The project focuses on substrate determination through a combination of *in vitro* assays and X-ray crystallography mapping out the individual chemical building blocks that the NRPSs use to synthesize their products. If successful we will attempt to synthesize the final product with a full-length NRPS construct and characterize it. Substrate specificity is generally associated with the adenylation domain of the NRPS which activates the substrate through expenditure of ATP hence this domain is a focus of our project in determining the products of the full-length NRPSs. Experiments have so far yielded substrate candidates for a number of the adenylation domains furthermore we have also determined the structure of one of the adenylation domains with AMP and the amino acid substrate bound in the active site. This represents the first structure of an archaeal NRPS domain paving the way for a deeper understanding of the similarities and differences between the archaeal NRPSs and eubacterial and eukaryotic NRPSs as well as ultimately elucidating the role of NRPSs in *Methanobrevibacter*.

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P48

Does HisG$_S$ really need it's friend?

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ATP-PRTase catalyses the first committed step of histidine biosynthesis in archaea, bacteria, fungi, and plants. As the catalyst of an energetically expensive pathway ATP-PRTase is subject to a sophisticated, multilevel regulatory system. Two types of ATP-PRTase have been reported; the long form (HisG$_{L)}$ and the short form (HisG$_S$), that differ in their molecular architecture.[1,2] A single HisG$_L$ chain comprises three domains. Domains I and II house the active site of HisG$_L$ while domain III forms the binding site for histidine as an allosteric inhibitor. The long form ATP-PRTase adopts a homo-hexameric quaternary structure. HisG$_S$ comprises a similar catalytic core to HisG$_L$ but is devoid of the regulatory domain and associates with a second protein, HisZ, to form a hetero-octameric complex.

Previous research has suggested that HisG$_S$ is devoid of catalytic activity in the absence of HisZ.[3] Here we reveal that HisG$_S$ exists as a marginally active dimer and catalysis is enhanced by the *in vitro* reconstitution of the hetero-octameric complex. We also report a large conformational change of the protein upon histidine binding to the HisZ subunit that illuminates a potential mechanism for the allosteric inhibition of the short form ATP-PRTase. In addition, we report the first characterisation of a third ATP-PRTase architecture in which HisZ and HisG$_S$ are covalently linked and assemble as a homo-tetramer.

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P49

Cofactor engineering of a clostridial alcohol dehydrogenase leads to unexpected changes in substrate specificity.

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The use of bacteria for the synthesis of useful chemicals typically derived from petroleum is a promising alternative to traditional production routes. Furthermore, synthetic biology can be employed to modify bacterial metabolism for the improvement of product yields. In this work we aimed to use mutagenesis to switch the cofactor specificity of the primary-secondary alcohol dehydrogenase from *Clostridium autoethanogenum*, a bacterium with considerable promise for the bio-manufacturing of fuels and other petrochemicals, from strictly NADPH-dependent to NADH-dependent[1]. As NADH is typically the more abundant intracellular metabolite we hypothesized this would lead to a more efficient enzyme *in vivo*, consequently increasing product output.

Initially we used a homology model to direct our rational design efforts. A serine residue at position 199 was identified as crucial for allowing the 2´-phosphate moiety of NADPH to bind. In an attempt to disrupt this binding, we built a site-saturation library focussed on this residue. We developed a 96-well plate screen based on the reduction of nitroblue tetrazolium to formazan dye in the presence of NAD(P)H to probe for variants showing activity with NADH, using acetone as the substrate. Unfortunately this library did not contain any NADH-utilising variants. As we had shown in previous work that this enzyme has a broad substrate range[2] we also tested this library with NADPH and the additional substrates butanone and acetoin and found that changes in the phosphate-binding site caused alterations at the substrate-binding site, despite a distance of 20 Å between the two. In particular, the S199A variant had a 300% increase in activity for the reduction of butanone to 2-butanol. Ultimately we used consensus-guided site-directed mutagenesis to identify four amino acid substitutions (G198D, S199G, R200G, and Y218F) that caused the desired switch in cofactor-specificity.

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2. Kopke, M., Gerth, M. L., Maddock, D. J., Mueller, A. P., Liew, F., Simpson, S. D., & Patrick, W. M. (2014). *Reconstruction of an acetogenic 2,3-butanediol pathway involving a novel NADPH-dependent primary-secondary alcohol dehydrogenase*. Applied and Environmental Microbiology, 80(11), 3394-3403.

P50

Understanding how *Phytophthora* ’sniff out’ their hosts

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Phytophthora are fungus-like microorganisms (oomycetes) that cause root rot and dieback diseases in thousands of plant species around the world. In New Zealand two important pathogens are *Phytophthora agathidicida*, which causes kauri dieback; and *P. cinnamomi*, which causes avocado root rot.

For many plant pathogens, including *Phytophthora*, chemotaxis (*i.e.* the ability to detect and navigate towards chemical signals) is critical for successful colonisation of the host. *Phytophthora* produce motile zoospores that are able to travel large distances through water-logged soils. Once they have located a host, they attach to its root surface and initiate infection. However, for most *Phytophthora* species, neither what they detect, nor how they detect it, is understood.

In this research, we are identifying the chemical signals that attract the zoospores to their plant host using qualitative and quantitative capillary assays. In addition, we are characterising one of the putative receptors that mediates this process. It has been suggested that *Phytophthora* use G-protein coupled receptors (GPCRs) that possess a C-terminal phosphatidylinositol phosphate kinase (PIPK) domain, to mediate chemotaxis.
Progress towards the expression and functional characterisation of a GPCR-PIPK from *P. agathidicida* will be presented.

P51

A high-throughput screen for ligand binding reveals three amino acid chemoreceptors from *Pseudomonas syringae* pv. *actinidiae*

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Anyone who has swatted away a hungry mosquito - or followed their own nose towards fresh coffee - will appreciate the usefulness of chemosensory cues for insects and mammals. Microorganisms also display surprisingly sophisticated sensory behaviours. By monitoring changes in the chemical composition of their environment, microbes move towards chemo-attractants (*e.g.* food sources) and flee from chemo-repellents (*e.g.* noxious chemicals and toxins). This process, termed chemotaxis, allows microbes to navigate towards more favourable environments. For many microbial pathogens, chemotaxis is a critical part of host invasion and colonisation.

We have begun to explore the structural and functional diversity of the chemoreceptors from *Pseudomonas syringae* pv*. actinidiae* (Psa). Psa is best known as the pathogen responsible for recent kiwifruit losses in New Zealand. It also has an unusually diverse chemosensory system, with 43 putative chemoreceptors encoded in its genome, none of which have been previously characterized. We developed a high-throughput fluorescence-based thermal shift assay for identifying the ligands recognized by a given chemoreceptor[1]. Using this assay, we screened 3 Psa chemoreceptors against 95 potential ligands. We found that each chemoreceptor recognized a distinct subset of amino acids. We further characterised one of these receptors, named *pscA*, using isothermal titration calorimetry, site-directed mutagenesis and chemotaxis assays.

1. McKellar, J., Minnell, J. and Gerth, M. (2015). *A high-throughput screen for ligand binding reveals the specificities of three amino acid chemoreceptors from Pseudomonas syringae pv. actinidiae*. Molecular Microbiology. 96:694-707.

P52

A plant-like PPR protein controls RNA cleavage in the *Plasmodium* remnant chloroplast

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The malaria parasite, *Plasmodium sp*. is one of the world's deadliest pathogens, around 200 million people are infected every year, resulting in 655 000 deaths, mainly in children under 5. Interestingly, this intra-cellular parasite has a photosynthetic ancestry and retains a remnant chloroplast termed the apicoplast. Although no longer photosynthetic the apicoplast carries out essential biochemical functions and is a target for existing anti-malarial drugs. As with the plant chloroplast, the apicoplast has its own (although greatly reduced) genome. This 35 kb genome contains around 60 genes, most of which are required for transcription and translation of the genome. Transcription within the apicoplast is polycistronic and characterisation of transcripts using circularised RNA identified potential processing sites. We have identified several nuclear encoded proteins in *Plasmodium* that are predicted to be apicoplast targeted that play important roles in transcription and post-transcriptional processing. One of which belongs to the pentatricopeptide repeat (PPR) protein family, which play essential roles in RNA stabilisation, splicing and editing in the plant chloroplast. We have characterised the PPR protein from *Plasmodium falciparum* and show it to be localised to the apicoplast and bind to specific RNA sequences at the identified processing sites to protect transcripts from degradation. The unique 'plant-like' nature of the PPR proteins could provide an avenue for the development of future anti-malarial drugs to combat emerging drug resistance to current therapies.

P53

Long vs Short; Transition state analysis of adenosine triphosphate phosphoribosyl transferases.

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The first committed step in histidine biosynthesis is catalysed by adenosine triphosphate phosphoribosyl transferase (ATP-PRTase). This pathway is energetically expensive and to control production at the enzyme level ATP-PRTase is regulated at the active site by AMP and allosterically by histidine. ATP-PRTase is found as two structurally diverse enzymes which differ in their allosteric machinery. The long form, HisGL, is a homohexamer with the subunit comprised of a mixed +/- bilobal domain containing the active site and a C-terminal ACT domain where the allosteric binding site is found. The short form is a hetero octomer, containing a similar mixed +/- bilobal catalytic core (HisGS) but instead of a regulatory ACT domain, HisGS associates with a second enzyme called HisZ to achieve allosteric regulation. Although structurally different, both HisGL and HisGS catalyse the same condensation reaction between PRPP and ATP, where the ribose-5-phosphate is transferred onto the N-1 of ATP to give PRATP (Figure 1.).

To investigate how the different regulatory domains affect the reaction at a mechanistic level we set out to determine the transition state of the two enzymes by measuring the intrinsic kinetic isotope effect (KIE) for Camphylobactor jejuni (HisGL) and Lactococcus lactis (HisGSZ). In this presentation the work towards making the labelled substrates through a coupled enzyme synthesis, how to measure the intrinsic KIE, and computing the transition states will be discussed.

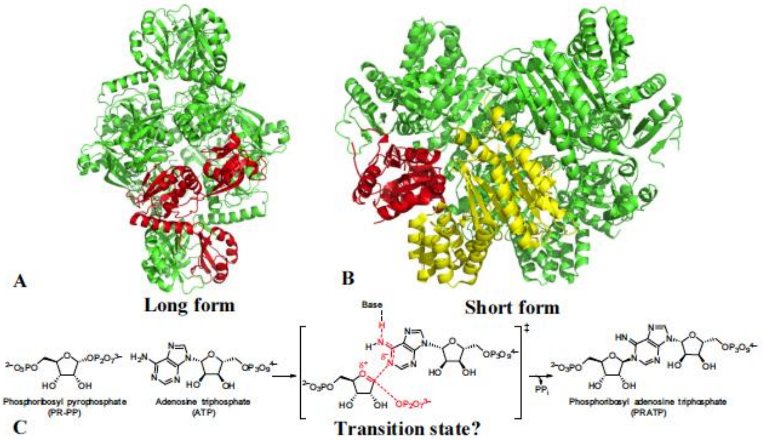

Figure 1. **A** The HisG$_L$ hexamer with in red the monomeric component. **B** The HisG$_S$Z octamer with in red HisG$_S$ and in yellow HisZ. **C** schematic overview of the catalyzed reaction.

P54

Engineering biofilm-blocking enzymes

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Quorum quenching is a strategy proposed to combat biofilms formed by bacterial pathogens, which can persist for long periods of time and are detrimental to human health. It inhibits quorum sensing, a chemical signal-based system used by a variety of pathogenic bacteria to detect population density and mediate group behaviour for processes such as biofilm formation and virulence. In contrast to traditional antibiotics, quorum quenching is thought to exert less selective pressure to develop resistance, as there is no immediate life or death selection. It is therefore a promising new approach to antimicrobial therapies.

Most Gram-negative bacteria, including pathogens such as *Pseudomonas aeruginosa*, *Burkholderia* and *Acinetobacter* species, use acyl-homoserine lactones (AHLs) as signal molecules for quorum sensing. We are interested in developing quorum-quenching enzymes to degrade these signalling molecules. AHL acylases are a class of enzymes that degrade AHLs by irreversibly cleaving them into their respective fatty acid and homoserine lactone products. However, the few AHL acylases characterised to date typically have poor heterologous expression and low yields.

Thus, I am using the glutaryl-7-aminocephalosporanic acid (GL7-ACA) acylase from *Pseudomonas* sp. SY77 as a template to engineer AHL acylase activity as it is a very well-characterised enzyme that is highly expressed in *Escherichia coli*. The GL7-ACA acylase has different substrate specificity to AHL acylases. However, these acylases share a similar structure and have conserved catalytic residues. I have characterised the activity of GL7-ACA acylase against GL7-ACA, its known substrate. Results of rational design and directed evolution strategies to develop AHL acylase activity from this template will be presented as well as development of assays for high-throughput screening of variant libraries.

P55

Investigating the peroxidase reaction mechanism of human cytochrome c.

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Cytochrome *c* is essential for ATP production and has a role in apoptosis. Cytochrome *c* has peroxidase activity which is believed to help trigger apoptosis. However, cytochrome *c* has a different active site compared to other haem peroxidases and the reaction mechanism is unclear. In 2008, we reported the first naturally occurring mutation in cytochrome *c* (G41S) in a NZ family with mild autosomal dominant thrombocytopenia[1]. Both the G41S mutation and recombinant G41A and G41T mutations increase the peroxidase activity of cytochrome *c*[2]. We hypothesise that by studying these proteins we will define the chemistry of the peroxidase reaction and the mechanism by which mutation of residue 41 increases activity.

Cytochrome *c* catalyses H_2O_2-dependent oxidation of various substrates. To gain insights into the peroxidase mechanism, we determined the effect of H_2O_2 on cytochrome *c*. H_2O_2 causes degradation of cytochrome c as monitored by decrease in Soret band absorbance (410 nm) and iron release. The rate of loss of the soret band and iron was highest for the G41T variant (G41T > G41S > G41A > WT). To investigate the mechanism of haem loss we undertook LCMS/MS analysis. This showed oxidation of the axial coordination ligand, Met80. H_2O_2-induced loss of axial coordination was confirmed by monitoring the 695 nm absorbance band, which depicts coordination between Met80 and Fe(III). Interestingly the presence of a small molecule substrate protected the protein from H_2O_2-induced damage. Taken together these results suggest that a cascade of events takes place in the vicinity of the haem in response to H_2O_2, and that mutation of cytochrome c at residue 41 increases accessibility of the haem to H_2O_2. Next, we will use Mössbauer spectroscopy to investigate the iron oxidation state, and determine whether the same changes occur in the presence of the physiological substrate cardiolipin.

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P56

Pinpointing the divergence of quaternary structure in the lysine biosynthetic pathway

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While quaternary structure is highly conserved among protein families, few studies have investigated the evolutionary history of enzyme complexes. In the work described here, we describe the evolution of quaternary structure in the lysine biosynthetic pathway. We have previously shown that there are significant differences in quaternary structure between the DHDPS (dihydrodipicolinate synthase) and DHDPR (dihydrodipicolinate reductase) enzymes of plants and bacteria. While both plant and bacterial DHDPS enzymes are mostly tetrameric, the arrangement of the subunits differs. Similarly, while bacterial DHDPR enzymes are tetrameric, plant DHDPR enzymes characterised are all dimers. Here, we present the first crystallographic data of dimeric DHDPR enzymes. In an effort to expand the knowledge of when these enzymes evolved to have different quaternary structures, we have characterised the structure and activity of DHDPS and DHDPR enzymes from moss, green algae, and red algae. We have found that while DHDPS from green algae has a similar structure to plants, red algal DHDPS has a similar structure to bacteria. Moss DHDPS has a novel arrangement in which it can form either dimers or tetramers, depending on ligand binding. Conversely, both red algal and green algal DHDPR enzymes appear to have dimeric structures, which are similar to that observed in plants. This work helps provide a better understanding of some of the evolutionary pressures behind self-assembly.

P57

Biophysical characterisation of chemotaxis receptor-ligand interactions from the Kiwifruit bacterial pathogen *Pseudomonas syringae pv. actinidiae*

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Pseudomonas syringae pv. actinidiae (*Psa*) is a bacterial pathogen that infects kiwifruit vines and has had a devastating effect on New Zealand fruit production in the last decade. A key element of *Psa* pathogenicity is the ability of the bacterium to respond to environmental stimulus. Chemotaxis allows the bacterium to sense chemical gradients and modulate their movement towards a favourable environment. Chemotaxis is mediated through the binding of chemicals to the chemoreceptors embedded within the bacterial membrane. The genome of *Psa* encodes 43 predicted chemotaxis receptors, of which only three have been characterised[1].

Homology modelling has suggested that the ligand-binding domains of two of the *Psa* chemoreceptors, 13100 and 20080, have CACHE-2 folds. The CACHE fold is one of the structures commonly found in chemotaxis receptors, however this fold binds a diverse range of chemicals. Therefore, in this study we aim to use a high throughput approach to identify and characterise receptor-ligand interactions.

The ligand-binding domains of *Psa* chemoreceptors 13100 and 20080 were recombinantly expressed in and purified from Escherichia coli Bl21(DE3). High throughput ligand screening was performed by fluorescence thermal shift assay and interactions were confirmed and by isothermal titration calorimetry (ITC)[1].

Thermal shift assays and ITC indicated that chemoreceptor 13100 interacts with small acidic ligands, such as propionic acid, acetic acid, pyruvic acid and glyoxylic acid. These receptor-ligand interactions and those of 20080 will be further characterised by X-ray crystallography.

1. McKellar, J., Minnell, J. and Gerth, M. 2015. A high-throughput screen for ligand binding reveals the specificities of three amino acid chemoreceptors from *Pseudomonas syringae pv. actinidiae*. *Molecular Microbiology.*

P58

Engineering an optimised selection system for folded proteins

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In many areas of research, the pursuit of folded, or soluble protein is a rate limiting step; from protein engineering to structural genomics, the ability to select a folded protein from a background of unfolded is extremely useful. Here we test and enhance the effectiveness of the pSALect system, which utilises the twin-arginine translocation (Tat) pathway to impart selection for folded proteins. The Tat pathway utilises specific N-terminal signal peptides to selectively transport folded proteins into the bacterial periplasm. Some signal peptides can lead to promiscuous exportation of unfolded proteins via the Sec translocation pathway. We utilised a Tat knockout strain of Escherichia coli to test the promiscuity of three Tat signal peptides which we have engineered within the two pSALect constructs, pSALect-fseI and pSALect-notI. We show that the charge at the C-terminal end of the signal peptide is important for transport specificity, as well as seeing significant differences between pSALect-fseI and pSALect-notI constructs with the same signal peptides. Furthermore we show that our engineered pSALect constructs are able to select for folded chimeras, while selecting against unfolded chimeras, in true positive and true negative testing. The results highlight that pSALect, especially in combination with our optimised Tat signal peptide, is an effective tool for in vivo protein folding selection, and could be very useful for screening protein libraries for folded constructs.

P59

Structural vaccinology of Group A Streptococcus Pili

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Background: Group A Streptococcus (GAS) causes a broad range of human disease ranging from benign pharyngitis and skin infections to severe invasive disease and the non-suppurative sequelae acute rheumatic fever (ARF) and rheumatic heart disease. Although ARF is now rare in most developed countries it causes significant disease burden in developing countries and the indigenous populations of some high-income countries. Maori and Pacific Island children in New Zealand have amongst the highest incidence of ARF in the developed world. There are no safe and effective vaccines against GAS currently available. This PhD project will lay the foundations for the development of a new GAS vaccine candidate based on the T-antigen (which forms the backbone of GAS pili) by probing the interactions between antibodies and T-antigens. Mapping the antibody binding sites and identifying protective epitopes for a vaccine candidate is an essential step in any rational vaccine design effort.

Methods: We will identify and map the binding sites of antibodies on T-antigens using antibody phage display. Phage-antibody repertoires will be screened to isolate monoclonal antibodies that are highly specific for a particular T-antigen. Strain specificity and cross-reactivity of the antibodies will be assessed using immunoassays and binding affinity will be measured using surface plasmon resonance. The epitopes will be mapped using a range of methods including x-ray crystallography, peptide tiling and peptide phage display. The protective capacity of the T-antigen specific antibodies will be measured using in vitro opsonophagocytic assays.

Outlook: This project will allow a structural map of protective T-antigen epitopes to be defined that will inform the design of a T-antigen based vaccine.

P60

Expression and purification of Keratin Associated Proteins for structural and biophysical characterization

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[2]AgResearch Limited, Lincoln Research Centre, Christchurch, New Zealand.

Keratin and keratin-associated proteins (KAPs) are the two major constituents of hair fibres, produced by the cortical cells of the hair follicle. Keratins belong to the intermediate filament (IF) protein family and are highly structured proteins. Keratins exist as native heterodimers, which are the building blocks that associate into higher-order molecular assemblies: intermediate filaments and macrofibrils. KAPs form a large family of proteins of varying sizes (92 different proteins, from 44aa to 517aa) that have atypical sequence features; many have low complexity sequences and contain repeated motifs. KAPs are classified into three different groups: ultra high sulfur (UHS) KAPs, high sulfur (HS) KAPs and high glycine-tyrosine (HGT) KAPs. They are the major constituents of the matrix between hair keratin IFs bundles and are known to interact with the head and tail domain of hair keratin.

The aim of the project is to increase our knowledge of the contribution of KAPs to hair fibre formation. Indeed, the molecular details of the interaction between keratins and KAPs are currently unknown. We have chosen to focus on KAP 11.1 (HS family) and KAP6.1 (HGT family) due to their expression profile in cortical cells. Their expression, purification and preliminary biophysical characterization will be presented.

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P61

Development of positive selection methods to improve nitroreductase activity by directed evolution

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Bacterial nitroreductases are members of a diverse family of oxidoreductase enzymes that are capable of activating nitroaromatic compounds, including anticancer prodrugs such as CB 1954 and PR-104A. This capability is useful in the anti-cancer gene therapy strategy known as gene-directed enzyme prodrug therapy (GDEPT), which involves the selective killing of tumour cells through activation of an inert prodrug to its cytotoxic form. One of the most promising nitroaromatic prodrugs, PR-104A, recently failed Phase II clinical trials as a hypoxia-activated (i.e., GDEPT-independent) prodrug due to off-target activation by the human enzyme AKR1C3 limiting the maximum tolerated dose. For GDEPT, it is therefore necessary to engineer more efficient nitroreductases to allow for a lower dose of prodrug to be administered, thus limiting unwanted side-effects. The use of random or targeted mutagenesis for development of more efficient enzymes leads to large libraries of variants, of which the vast majority are non-functional. To allow efficient screening of these libraries, we have developed an array of genetic and biochemical methods to enable the rapid selection of active nitroreductases from the pool. Here we have investigated the effectiveness of these different approaches for improving nitroreductase activity, and how effective they are at improving activity with specific target substrates such as PR-104A.

P62

Directed Evolution of Bacterial Enzymes to Create Powerful Tools for Cellular Ablation Studies

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Bacterial nitroreductase enzymes (NTRs) are proving an important tool in ablation studies that aim to elucidate mechanisms of tissue regeneration and cell lineage relationships. When expressed in a cell an NTR can act as a molecular switch, reducing an otherwise innocuous prodrug to a cytotoxic product that induces cell death. In transgenic zebrafish, tissue-specific promoters enable the expression of an NTR within specific cell populations. Following systemic administration of a prodrug, these cells can be specifically ablated. Analysis of cell recovery after ablation may reveal novel cellular and molecular mechanisms underlying the regeneration process, thus bringing new insights to the field of regenerative medicine.

Our work seeks to expand the utility of this system by engineering a pair of NTRs to have non-overlapping prodrug specificity, to allow researchers to specifically ablate two different cell populations within a single organism in a spatially and temporally controlled manner. A library of mutant NTRs was generated by randomising residues putatively associated with substrate binding in the NTR active site. The library was concurrently screened for loss of activity with 5-nitroimidazole prodrugs such as metronidazole, and retention of activity with 2-nitroimidazole prodrugs such as benznidazole. These screens revealed two promising NTR variants, one of which is active only with metronidazole, and another that is active only with benznidazole. To date, proof-of-principle testing has been conducted *in vivo* in bacteria cells and future experiments will involve examining whether this activity profile carries through when each NTR is expressed *in vivo* in eukaryotic cells.

P63

Reconstruction of Ancestral Enzymes from a Core Biosynthetic Pathway

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The reconstruction of ancestral enzymes from sequences of contemporary proteins (known as ASR) is a beneficial tool in the elucidation of properties of ancient enzymes and, by extension, organisms. ASR has been used to understand a variety of core proteins in processes such as metabolism[1] and homeostasis[2]. The Shikimate pathway, involved in the biosynthesis of key amino acids tryptophan, phenylalanine and tyrosine, is an ideal candidate for use of ASR in order to understand the evolutionary history behind its development. We have inferred two candidate Shikimate enzymes from the last common ancestor of the Bacillus bacterial genera that are present in the aroFBH operon of Bacillus subtilis using a robust ASR method that utilizes species-aware phylogenetic trees[3]. These enzymes will be expressed and purified, along with their contemporary counterparts from B.subtilis, to be used in experiments to assess their biophysical and biochemical properties. This includes comparative assays of rate and the use of molecular dynamics to predict intra- and inter-molecular interactions. The contemporary shikimate enzymes will be replaced with their ancestors in B.subtilis in order to ascertain how complicated core metabolic pathways, such as amino acid biosynthesis, may evolve. We aim to extend this methodology to all seven enzymes of the shikimate pathway in order to reconstruct the entire biosynthetic pathway.

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P64

Design and optimisation of a screen to enable directed evolution of superior DNA ligases

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A novel screening approach has been designed for DNA ligase activity, intended for application in a future directed evolution study. DNA ligase enzymes are among the most widely used and important enzymes in molecular biology. They are becoming increasingly important in next-generation sequencing, however DNA ligases have evolved for a primary function of nick repair, and naturally occurring ligases are very inefficient at catalysing the blunt-ended ligations that underpin next-generation sequencing technologies. DNA ligase enzymes fused to DNA binding proteins have previously been developed with the aim of increasing enzyme affinity for the DNA substrate, and were significantly more effective at catalysing blunt ended ligations than the most commonly used DNA ligases from T4 bacteriophage and *Escherichia coli* [1]. To achieve further improvements required to bring a product to market, we have designed, tested and optimised a selection protocol to enable DNA ligase activity to be improved by directed evolution – a process of random mutagenesis followed by selection for improved enzyme variants. This selection involves using a bacterial cell that contains a plasmid encoding a DNA ligase gene variant as both a source of DNA ligase enzyme, and as a source of template to produce linearised plasmid DNA via PCR as substrate for the ligation reaction. In brief, the DNA ligase enzyme variant acts on its own plasmid, recircularising a linear form of the plasmid to seal a dsDNA break in the ampicillin resistance gene. The DNA ligase enzyme variants that are more efficient at catalysing this ligation step generate the highest number of complete plasmid copies. Recircularised plasmids are selected post-transformation into *E. coli* and exposure to ampicillin. Over multiple rounds of screening plasmids containing the most efficient DNA ligase gene variants will be selected for, allowing enrichment of genes encoding the enzymes that are most efficient at blunt ended ligation.

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P65

The structure of a rumen bacteria glycoside hydrolase 29 family αLfucosidase reveals unique dual carbohydrate binding domains

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The glycoside hydrolase (GH) family 29 consists solely of α-L-fucosidases. These enzymes catalyse the hydrolysis of glycosidic bonds. To date only five X-ray structures of GH29 enzymes have been deposited in the Protein Data Bank (PDB). We have solved the structure of 0940_GH29, a protein cloned from metagenomic DNA from the rumen of the cow, which reveals a multi-domain arrangement that has not been seen before in GH29 enzymes. The microbial source of this enzyme is unknown. This enzyme includes an additional carbohydrate binding domain at the Cterminal end as well as the typical N-terminal catalytic domain and carbohydrate binding domain. In comparison to other GH29 enzymes, 0940_GH is a monomer and its overall structure consists of an Nterminal TIM barrel like domain, a β-sandwich domain and a Cterminal β-sandwich domain. The TIM barrel like catalytic domain exhibits a $(\beta/\alpha)_7$ arrangement instead of the typical $(\beta/\alpha)_8$ design with the “missing” secondary structures replaced with a long meandering loop that “closes” the barrel structure and suggests a high degree of structural flexibility in the catalytic core. The two binding domains are classed as family 32 carbohydrate binding modules (CBM32). These domains have residues involved in ligand binding in the loop regions at the edge of the β-sandwich. The residues predicted to be involved in substrate binding differ between the modules, suggesting that different modules bind to different substrates. Enzymes that possess multiple copies of CBMs are thought to have a complex mechanism of ligand recognition. The additional C-terminal CBM32 is positioned alongside the catalytic domain, facilitated by a long hydrophilic loop from the preceding CBM32. This suggests that this domain may have a dynamic range of movement enabled by the loop, allowing a unique mode of action for a GH29 enzyme not identified previously.

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Structural Characterisation of Menangle virus Nucleocapsid-like Particles

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Paramyxoviruses infect many animals including mammals, birds, reptiles and fish. Common human pathogens such as the measles, mumps, and parainfluenza viruses belong to this large family of viruses. The single-stranded negative-sense RNA genome of these viruses is packaged by their nucleocapsid protein into a helical complex, termed the nucleocapsid.

During RNA synthesis the viral polymerase must engage and move along the nucleocapsid. Our research is focused on characterising polymerase-nucleocapsid interactions, and the mechanisms governing polymerase translocation during RNA synthesis. This work requires a detailed structural model of the nucleocapsid.

Here we report the structural characterisation of particles modelling the nucleocapsid of Menangle virus (MenV), a zoonotic bat-borne Paramyxovirus, which is closely related to human mumps virus. These nucleocapsid-like particles (NLPs) have 13 subunits arranged into a ring with cyclic symmetry. Each ring corresponds to one turn of the helical nucleocapsid. Spectroscopic methods were used to show that the rings are loaded with RNA. Using Cryo-electron microscopy and single particle 3D reconstruction, we have produced a low-resolution structure of the ring-like NLPs. Interestingly, structural analysis reveals the rings have a propensity to form two-ring disks, with the rings in each disk packed in opposing orientations. Small angle X-ray scattering experiments and analytical ultra-centrifugation data have confirmed the major species in solution to be a two-ring disk.

P67

Investigation of a putative proton relay chain in the enzyme 3-deoxy-D-*manno*-octulosonate 8-phosphate synthase

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3-Deoxy-D-*manno*-octulosonate 8-phosphate synthase (KDO8PS) is only expressed in plants and Gram-negative bacteria, where it is involved in the lipopolysaccharide biosynthesis pathway. [1] KDO8PS is closely related to the enzyme 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase (DAH7PS), which catalyses the first step of the shikimate pathway.

KDO8PS catalyses the condensation of arabinose 5-phosphate (A5P) and phospho*enol*pyruvate (PEP) to form 3-deoxy-D-*manno*-octulosonate 8-phosphate (KDO8P).[2] In contrast DAH7PS catalyses the reaction between PEP and erythrose 4-phosphate (E4P). Many of the key steps of these reactions have been previously determined.

There are slight differences between the active-sites of KDO8PS and DAH7PS. In DAH7PS there is a conserved glutamate residue, which in KDO8PS is conserved as an aspartate residue. In DAH7PS, the catalytic glutamate residue has been suggested to take part in a putative proton relay chain critical for the catalytic mechanism.

We have examined the role of the conserved active site aspartate in KDO8PS by examining variants where this residue and a neighbouring proline residue are substituted. The functional properties of these variants and their structures shed light on both the catalytic mechanism of KDO8PS and the evolutionary relationship between KDO8PS and DAH7PS.

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P68

Engineering Quorum Quenching Enzymes to Prevent *Pseudomonas aeruginosa* Biofilm Formation

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Pathogenic bacteria such as *Pseudomonas aeruginosa* use a method of communication commonly known as quorum sensing to regulate biofilm formation and establish chronic infections. Quorum sensing is mediated by the concentration of small diffusible signalling molecules, the concentration of which is dependent on the local population density. This process enables bacteria to replicate without displaying virulent behaviour until a population capable of overwhelming the host defences is reached. Disruption of this system – commonly referred to as quorum quenching – has been shown to be an effective method for preventing biofilm formation. The disruption of biofilm formation has the potential to reduce pathogenicity of many organisms, without promoting the development of antibiotic resistance.

Several quorum-quenching enzymes have been identified, including HacB, an acyl-homoserine lactone (AHL)-acylase enzyme from *P. aeruginosa* PAO1. AHLs are the major quorum sensing molecules used by Gram-negative bacteria. Specificity of these molecules is determined by the length of the fatty-acyl chain. AHL-acylases interfere with quorum sensing by irreversibly degrading AHLs via the hydrolysis of an amide bond. HacB specifically hydrolyses the long chain AHLs (C8-, C10-). *P. aeruginosa* uses both a long chain (3-oxo-C12-) and short chain (C4-) homoserine lactone (HSL) dependent quorum sensing system. Thus, if mutagenesis approaches can alter the substrate specificity of HacB, there is the potential to prevent *P. aeruginosa* biofilm formation.

Using rational design, I have successfully altered the substrate specificity of HacB. Using a luminescence-based reporter assay I characterised the mutants constructed and found one capable of hydrolysing C4-HSL. This activity has previously not been observed in other AHL-acylases. I am currently in the process of determining the ability of these variants to prevent and/or disrupt the formation of *P. aeruginosa* PAO1 biofilms.

P69

The structure of Cas1 and its role in adaptation in Type I-F CRISPR-Cas systems

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CRISPR-Cas are adaptive immune systems found in many prokaryotes that confer resistance against mobile genetic elements (MGEs) such as bacteriophage and plasmids. There are many different types and subtypes of CRISPR-Cas systems, but all are characterised by a CRISPR array containing a bank of short sequences (spacers) derived from invading elements. Upon infection with an MGE, this CRISPR array can be transcribed and processed into many short CRISPR RNAs, each containing a single spacer, which are then loaded into surveillance complexes of Cas proteins. These large ribonucleoprotein complexes then identify and destroy targets that match the spacer in the CRISPR RNA. However, if a prokaryote is infected with a MGE that it has not previously encountered, adaptation can occur, where a new spacer derived from the MGE is incorporated into the CRISPR array, thus "immunising" the host. Adaptation is poorly understood at a biochemical level, but is known to require a complex of the universally conserved Cas proteins Cas1 and Cas2. Here we characterise the biochemistry of adaptation in the Type I-F CRISPR-Cas system of the potato pathogen *Pectobacterium atrosepticum*. Type I-F systems are characterised by a unique fusion of the adaptation protein Cas2 with Cas3, the protein responsible for target destruction, and this fusion is known to form an adaptation complex with Cas1. We solved the crystal structure of Cas1 and have been characterising the adaptation process *in vitro*.

P70

Evolving bacterial nitroreductase enzymes for improved activation of a challenging next-generation prodrug substrate

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There is strong interest in developing novel targeted cancer therapies such as viruses or bacteria that can preferentially infect and lyse cancerous cells while sparing surrounding healthy tissue. One strategy to enhance the potency of such targeted therapies is to “arm” the agents with an additional gene, the enzyme product of which can convert a systemically delivered benign prodrug into a potent chemotherapeutic. Crucially the activated chemotherapeutic should exhibit a “bystander effect”, i.e. be able to diffuse to and kill neighbouring cancer cells to maximise the therapeutic effect.

Bacterial nitroreductases are highly promiscuous enzymes. As part of a multi-institute collaboration we have exploited this to develop nitroreductases that can convert non-toxic nitroaromatic prodrugs into potent DNA alkylating cytotoxic agents. Next generation nitroaromatic prodrugs have been chemically engineered to optimize their pharmacokinetic and pharmacodynamic properties and cytotoxic potential - in particular to maximise their bystander effect. Unfortunately these chemical modifications have yielded poorer nitroreductase substrates, e.g. our top nitroreductase NfsA exhibits a 10 fold lower specificity constant with our lead next-generation prodrug than with our previous lead prodrug PR-104A. A primary impediment to nitroreductase activity appears to be a bulky piperazine side-chain. Four NfsA active site residues likely to be influencing accommodation of the piperazine moiety were identified by modelling, and a plasmid based library simultaneously targeting these residues via site saturation mutagenesis was cloned into *Escherichia coli*. The library was enriched for ~5000 active mutants by plating on solid agar containing niclosamide, a compound that is detoxified by nitroreduction and hence provides a powerful positive selection for nitroreductase activity. In ongoing work the top 10 hits from this library are being validated by purified protein kinetics. These results will then be used to select active site mutations to recombine with beneficial mutations identified in previous rounds of random mutagenesis.

P71

Snakin-1: a racemic success story

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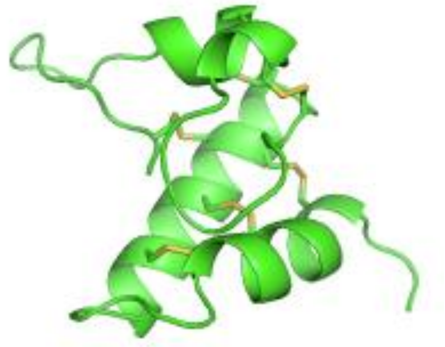
Snakin-1 is a 63 residue antimicrobial protein originally isolated from potato (Solanum tuberosum).[1] It is active against a number of bacterial and fungal phytopathogens such as *Clavibacter michiganensis*, *Pseudomonas syringae* and *Fusarium solani*. Snakin-1 is a member of the GASA (gibberellic acid stimulated in Arabidopsis)/snakin family and the mature protein consists of a GASA domain incorporating six intramolecular disulfide bonds.[2] The amino acid sequences of these proteins do not correspond to any known structural motifs. GASA/snakin proteins are found in a variety of plant species and appear to be involved in a range of functions including cell elongation and cell division.[2] Their expression profiles support these roles and are commonly linked to development.[2] It has also been speculated that the 12 conserved cysteines in these proteins perform a role in redox regulation.[2]

We have recently completed the total chemical synthesis of native Snakin-1 and showed that its antimicrobial activity is comparable to that of the naturally occurring protein.[3] In an attempt to understand how this small protein functions we have determined its threedimensional structure by X-ray crystallography using a quasi-racemic protein system.[4] Phase information for structural determination was obtained by radiation-damage induced phasing.[5]

The structure of snakin-1 appears to be novel, different to known classes of cysteine-rich plant antimicrobial peptide. Its features include a large and distinctly electropositive loop that we speculate to be membrane targeting, and a two helix bundle which is a potential membrane-interacting feature able to disrupt the structural integrity of its target bacteria.



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P72

Nano-LEGO: Making it CLICK!

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Peroxiredoxin proteins are key peroxidases found in all kingdoms of life. They can form a plethora of protein architectures: from monomers and dimers to rings of different subunit composition. These rings are also able to stack to form nano-tubes at lower pH[1]. Their impressive repertoire of assemblies make peroxiredoxins ideal building blocks, or nano-LEGO, for creating switchable nano-structures for extracellular applications.

In order to expand the functionalisable potential of peroxiredoxins, unnatural amino acids can be specifically incorporated into protein sequences. In particular, the unnatural amino acid, p-azidophenylalanine, was built into human peroxiredoxin 3 protein to permit click chemistry at precise positions on the protein surface.

Characterisation of this modified protein is crucial to ensure their notable structure switching capabilities is retained, thus validating peroxiredoxins as versatile protein nano-LEGOs that can be easily functionalised.

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P73

Characterisation of RNF11 – a negative regulator of immune signalling

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The covalent attachment of a 76-residue protein, ubiquitin, to cellular proteins ('ubiquitylation') can regulate the abundance, activity, and sub-cellular localisation of the modified proteins. In turn, this can influence the outcome of cell signalling pathways[1]. Ubiquitylation occurs through a cascade involving three classes of enzymes: E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzymes, and E3 ubiquitin-protein ligases. The majority of E3 ligases possess a RING (Really Interesting New Gene) domain, which interacts with E2 enzymes and catalyse direct transfer of ubiquitin from the E2 to Lys residues on specific set of substrate proteins. Ubiquitin itself contains seven Lys residues and, therefore, can act as a substrate for subsequent attachment of ubiquitin molecules to generate poly-ubiquitin chains[2].

Tumour Necrosis Factor (TNF) Receptor-Associated Factor 6 (TRAF6) is a RING domain-containing E3 ligase, which together with the E2 enzyme Ubc13, activates pro-inflammatory signalling pathways by building poly-ubiquitin chains. These chains serve as recruitment platforms for multiple protein kinases[3]. We have reconstituted the poly-ubiquitin chain building process *in vitro* by using highly purified enzymes. In addition, we have begun to study the properties of another E3 ligase RNF11 (RING Finger 11 protein) using the same system. Surprisingly, although both E3 ligase bind Ubc13, RNF11 did not display significant chain-building activities. The possible implications of RNF11-Ubc13 interaction to immune signalling will be discussed.

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P74

Towards understanding the role of non-ribosomal peptide synthetases in methanogenic archaea

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Non-ribosomal peptide synthetases (NRPSs) are large enzymatic “assembly lines” that synthesise diverse bioactive non-ribosomal peptides. Non-ribosomal peptides include many antibiotics and other pharmacologically important molecules. NRPSs are common in eukaryotes and eubacteria but were not known in archaea until the first examples were recently discovered in methanogenic archaea living in the rumens of livestock.[1] Given the high energetic cost of producing the large NRPS enzymes (275-475 kDa) and the energy-limited lifestyle of the anaerobic methanogens, we expect that the non-ribosomal peptides play a critical role in methanogen biology. However, these archaeal NRPSs are not expressed in culture and nothing is currently known about the non-ribosomal peptides that they produce. We aim to use purified NRPS proteins to synthesise their non-ribosomal peptide products *in vitro* and characterise the synthesised products. NRPSs are known to synthesise non-ribosomal peptides from a diverse array of amino acid and carboxylic acid building blocks. Hence, we have been identifying the building blocks that are utilised by the archaeal NRPSs *via* biochemical and structural investigations of the specificity-conferring adenylation domains of the NRPSs. Our results have revealed that the archaeal NRPSs we have investigated to date have relaxed substrate specificities. We have also expressed and purified the full-length NRPS proteins in *Escherichia coli* and efforts to synthesise the non-ribosomal peptide products are on-going. This will allow us to begin to elucidate the role that these non-ribosomal peptides play in methanogen biology. Our research is expected to lead to novel targets for mitigating enteric methane emissions from livestock, which make up 30-35% of New Zealand’s total greenhouse gas emissions and a significant and increasing proportion of global emissions.

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