CBCB Symposium 2017, 5 July, Baddiley Clark Seminar Room

Abstracts

Professor David Holden FRS

Immune Interference by *Salmonella*

Dr Kevin Waldron

Key role of a 'promiscuous' metalloenzyme, able to switch metal cofactors, during *Staphylococcus aureus* infection

Unlike other *staphylococci, S. aureus* possess duplicate genes encoding the enzyme superoxide dismutase (SOD), an enzyme that plays a key role in detoxification of reactive oxygen species (ROS) during infection to counter the host's oxidative burst. Both of these SOD enzymes were predicted to utilise manganese as their essential redox-active cofactor, yet expression of the duplicated gene, sodM, is unexpectedly induced under Mn starvation conditions. Importantly, the host suppresses Mn availability to S. aureus during infection through the metal sequestration properties of the immune effector calprotectin, leading to diminution of total SOD activity. However, we found that SodM activity increases in S. aureus cells treated with calprotectin. This observation is explained by a remarkable property of the SodM enzyme; it is 'cambialistic', able to switch from using manganese to using iron as its essential cofactor, enabling S. aureus to maintain its pivotal defence against host-derived ROS during manganese starvation it experiences during infection.

Here, we will discuss some of our latest results characterising the role of the dual SODs of *S. aureus*. In vitro and in vivo evidence will be presented demonstrating the catalytic properties and the importance of the duplicate SOD enzyme. Biochemical and biophysical data on the two S. aureus SODs will show that these closely related and highly homologous proteins represent an excellent model system in which to determine how metal specificity of enzymatic catalysis is achieved.

Dr Alan Cartmell

Unusual active location and catalytic apparatus in a glycoside hydrolase family

The location of the active site of enzymes with the same fold is invariably conserved. The β-propeller fold exemplifies this feature with all functions located at what is termed their anterior surface. Herein, however, we show that the active site of a glycoside hydrolase that adopts the β-propeller fold is located to the posterior surface of the α-l-rhamnosidase. The enzyme also displays a catalytic apparatus that utilizes a single histidine instead of the canonical pair of carboxylate residues deployed by the vast majority of glycoside hydrolases. The capacity to engineer catalytic functionality into the posterior surface of other family members provides insight into the evolution of this enzyme family

Professor Nick Jakubovics

Biofilms: Life at the Interface

In nature, the vast majority of microorganisms live in structured polymicrobial communities, embedded in extracellular polymers and associated with interfaces. In the late 1970s, the term 'biofilm' was coined to describe these microbial aggregations. Bacteria in biofilms behave differently from their planktonic counterparts. Understanding biofilm biology is critical for controlling unwanted microorganisms in the environment or in the human body, or for exploiting microbes for beneficial goals. Surface colonization is the first step in biofilm formation and is mediated by interactions between bacterial cells and a chemical conditioning layer. Cell-cell interactions drive the development of surface-associated biofilms or the formation of aggregates, and bring genetically distinct cells into juxtaposition where they may exchange nutrients, signals or antagonistic products. The biofilm matrix encases cells, and traps small molecules in the local vicinity while retarding the penetration of antimicrobial agents. The field of biofilm research has attempted to define broadly applicable rules that govern the assembly and functioning of diverse microbial communities. Although much progress has been made, some of the fundamental biological characteristics of biofilms have yet to be defined.

Professor Colin Harwood

Early stages in *Bacillus subtilis* secretion; revisiting the Signal Recognition Particle (SRP)

The SRP is a ribonucleoprotein complex that is essential for protein secretion. However, much of what is currently understood about bacterial SRPs in general is derived from work on *Escherichia coli* and higher organisms. In contrast, few studies have focused specifically on the SRP of Gram-positive bacteria. This is significant because the structures of the Gram-positive and Gram-negative SRPs are signifcantly different, pointing to potential differences in their modes of action and activities. In particular, the lengths of their RNA components are different, 271nt in the case of *B. subtilis* and 114 nt in the case of E. coli. This means that while both have "S" domains, E. coli lacks an "Alu" domain present is the SRP's of all other domains of life.

Our work is aimed at gaining insights into structural and functional relationship of the *B. subtilis* SRP with the protein secretion pathway. To this end we use a combination of biophysical and genetic techniques to elucide the structural components of the *B. subtilis* SRP. The SRP was reconstructed in vitro and Electro-mobility Shift Assays (EMSA) and MicroScale Thermophoresis (MST) were used to analyse the binding affinities between RNA its protein components.
We have shown that the previously reported binding of Hbsu to the Alu domain is likely to be an experimental artefact since this protein only shows a weak interaction with the S domain, most likely due to its non-specific affinity for nucleic acids. In contrast, the product of the ylxM gene, located directly downstream of ffh on the B. subtilis chromosome, shows binding coefficients to Alu and S domains are 107.2 ± 15.20 and 50.5 ± 40.8 nM respectively.

In conclusion, we shown that YlxM, present in other Gram-positive species but not in Gram-negative species, is a component of the *B. subtilis* SRP. We discuss a model in which a structural resemblance of the Alu domain to tRNA means that, potentially, an interaction between the Alu domain, YlxM and EF-Tu could be responsible for bringing about translational arrest. The absence of this structure in Gram-negative bacteria means that an alternative arrest mechanism presumably exists in this group of organisms.

Dr Daniel Peters

Making skin from the plague: Engineering the Caf1 protein from *Yersinia pestis* for biomedical applications

Yersinia pestis, the causative agent of the plague, is able to protect itself from phagocytosis by host macrophages through the secretion of a gel-like coat composed of the Caf1 protein. Caf1 forms very long (um, MDa), thin, non-covalently linked polymers composed of ~15 kDa subunits. The polymers are assembled at the outer membrane through the chaperone-usher pathway, and are secreted from the bacterium to form the capsule.

Mammalian cells adhere poorly to Caf1 in vitro, but this phenotype can be reversed by incorporating the integrin binding motif, RGDS, into the protein's structure. Additionally, the protein is highly stable, can be produced through bacterial fermentation, and is animal-free. Therefore, Caf1 has the potential to be a valuable new cell scaffold biomaterial for use in 3D tissue culture, drug discovery, and wound healing.

Here, we present our efforts to develop Caf1 as a biomaterial, including the bioprocessing, stability measurements, and rheological characterization of the material. Additionally, we describe the rational design and engineering of the protein's functionality through the inclusion of binding motifs, protease sites and differentiation signals. Moreover, we demonstrate the use of synthetic biology techniques to produce mosaic polymers. These polymers contain more than one type of Caf1 functional subunit and more effectively mimic natural extracellular matrix proteins, such as fibronectin. The production of a highly stable, animal-free material of definable bioactivity should be of great use in biomedical applications, and further development will focus on increasing the range of functionalities that Caf1 possesses.

Professor Nikolay Zenkin

Dissecting mechanisms of transcription termination

Transcription on vast majority of bacterial genes is terminated by an RNA hairpin of the transcript preceding a poly-U signal that serves to pause transcription. We show that eukaryotic RNA polymerase I (pol I) uses a similar mechanism. Pol I synthetises the precursor of the ribosomal RNAs - the majority of RNA in eukaryotic cells. For termination of synthesis,

pol I is road-blocked by DNA binding protein Reb1 (rather than paused by poly-U like in bacteria). This stop was believed to be followed by endonucleolytic cleavage of the RNA behind pol I by Rnt1, and exonuleolytic degradation of the pol I-bound portion of nascent RNA by Rat1, resulting in displacement of pol I from the template via “torpedo” mechanism. By using nuclear lysates, purified pol I, promoter originated transcription and assembled elongation complexes, we show that efficient termination by pol I does not require rnt1 or rat1. We show that a large conserved RNA hairpin that forms in the nascent transcript behind road-blocked pol I is sufficient to cause displacement of pol I from the template. After collision with Reb1 road block, pol I backtracks to reach this hairpin, which causes termination likely by a mechanism similar that of bacteria. We suggest that this is one of several redundant mechanisms that act to ensure faithful termination by pol I to facilitate recycling of pol I and block detrimental readthrough into downstream genes. Our finding suggests an ancient nature of the hairpin-dependent termination of transcription, that evolved before divergence of bacteria and archaea/eukaryotes.

Dr Yann Cesbron

A close look at the spatio-temporal organisation of the bacterial cell divisome

Dr Javier Abellon-Ruiz

How to keep an asymmetric outer membrane in good health

The Gram-negative bacterial outer membrane (OM) is a unique bilayer that forms an efficient permeation barrier to protect the cell from noxious compounds such as antibiotics. The defining characteristic of the OM is extreme lipid asymmetry, with phospholipids comprising the inner leaflet and an outer leaflet consisting of lipopolysaccharide (LPS). Asymmetry is crucial for OM barrier function, and the entropically favourable but deleterious presence of phospholipids in the outer leaflet causes susceptibility to many small molecules. OM lipid asymmetry is directly maintained by the Mla pathway, a six-component system that is widespread in Gram-negative bacteria and is thought to mediate retrograde transport of phospholipids from the OM to the cytoplasmic membrane. The OM lipoprotein MlaA performs the first step in this process via an unknown mechanism that does not require external energy input. In this talk I will present our latest results (using X-ray crystallography, molecular dynamics simulations and functional assays) about this first step showing that MlaA is a phospholipid translocation channel. MlaA is a monomeric integral OM protein composed almost exclusively of amphipathic α-helices, forming a ~20 Å thick donut embedded in the inner leaflet of the OM with a central, amphipathic pore. This architecture prevents access of inner leaflet phospholipids to the pore, but allows docking of outer leaflet phospholipids to an extracellular surface-exposed ridge surrounding the channel, followed by diffusion towards the periplasmic space. We also find that while Enterobacterial MlaA proteins form stable complexes with OmpF/C, the porins do not play an active role in phospholipid transport. Our data thus reveal that MlaA represents a novel class of lipid transport protein that selectively removes outer leaflet phospholipids to help maintain the essential barrier function of the bacterial OM.

Professor Waldemar Vollmer

Peptidoglycan synthesis in the test tube