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**Keywords: cytokine, receptor, structure**

## MS.36.2

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**Bacterial cell wall degradation by a staphylococcal autolysin**

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Treatment of hospital-acquired Staphylococcus infections remains a challenge as the number of strains with multiple antibiotic resistances has increased steadily over the last decades. This emphasizes the need for a new class of antibiotics that act on new targets. The major autolysins AtlE and AtlA of *S. epidermidis* and *S. aureus*, respectively, represent such targets. Both enzymes take over pivotal roles in cell division where they are responsible for the separation of daughter cells.

The high-resolution structure of AmiE reveals for the first time detailed insights into the enzymatic function of a staphylococcal peptidoglycan (PGN) hydrolase and thereby provides the basis for the formulation of a likely mechanism of catalysis [1]. With the synthesis of PGN fluorescent substrates, a method was established that offers the option to produce substrates of defined length and high purity [1,2]. Easy modifiability of the synthetic substrates also allows probing the influence of amino acid substitutions on substrate recognition. It could be demonstrated that the presence of a third amino acid in the peptide stem as well as the isoform of glutamine in the second position are key motifs and essential for recognition by AmiE. These findings could also be confirmed in a docking model. Structural comparisons with other proteins sharing the amidase-fold reveal common features in the substrate grooves, which points to an evolutionary conserved mechanism of PGN recognition.

Contrary to previous assumptions according to which the cell wall binding region (CBR) of the AtlE amidase contains only two repeat domains, the crystal structure of $R_{3,4}$ reveals the presence of four highly similar domains that belong to the family of SH3b domains. The sequence and structural similarity between every second repeat in the CBR is higher than between adjacent ones. A distinct patch of conserved residues could be located on opposite sides of each repeat in the $R_{3,4}$ tandem repeat. These areas might serve as binding pockets for negatively charged ligands such as teichoic acids. We are currently examining this possibility.

Small angle x-ray scattering (SAXS) experiments of AmiE-$R_{1-4}$ and $R_{1-4}$ reveal that the hinge region between the catalytic domain and the first repeat module is more flexible than the second one separating $R_{1,2}$ from $R_{3,4}$. In conjunction with the available structural data, it is now possible to draw a plausible mechanism of cell wall interaction in which the flexibility of the first linker ensures access of the catalytic domain to several PGN cleavage sites. The second linker serves to separate the two tandem repeats of the CBR. This creates a binding groove in $R_3$ that can easily be modulated by movement of the RT-loop. In comparison to its counterpart in $R_4$, the RT-loop of $R_3$ is not engaged in any contacts with the preceding domain.

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**Keywords: bacterial, catalysis, x-ray**

## MS.36.3

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**Gram-positive bacterial pili: structures, assembly and function**

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Bacterial pili are filamentous appendages that are critically involved in adhesion to host cells, leading to colonization of host tissues and establishment of infections. They are built from protein subunits called pilins; the backbone of the pilus is formed by hundreds of copies of a major pilin, while several minor pilins with specialized functions are associated with the backbone. While pili of Gram-negative bacteria such as *E. coli* have been extensively studied, the pili on Gram-positive organisms have been only recently discovered and are fundamentally different in that the pilins are held together by covalent isopeptide (amide) bonds formed by sortase enzymes.

We determined the first atomic structure of a Gram-positive major pilin, Spy0128 from *Streptococcus pyogenes* (Group A Streptococcus; GAS) [1]. The crystal structure showed an elongated molecule comprising two immunoglobulin (Ig)-like domains. The fold of each domain resembles the repeating CnaB domains of the collagen-binding adhesin Cna from *Staphylococcus aureus* and demonstrates a possible evolutionary relationship that links pili to a large family of cell surface proteins involved in binding to the extracellular matrix. In addition, the crystal structure brought two unexpected surprises that brought significantly new understanding of the assembly of Gram-positive bacterial pili. Firstly, the head-to-tail packing of successive molecules in the crystal provides a very persuasive model for pilus assembly, and mass spectrometry of native GAS pili validated this model. Secondly, the structure revealed a previously unknown mechanism for stabilizing proteins, in the form of self-generated intramolecular isopeptide (amide) bonds, which explains the long-known stability of Lancefield T-antigens. We also determined the high resolution crystal structure of a major pilin SpaA from *Corynebacterium diphtheriae* [2]. This reveals a similar modular structure of Ig-like domains, yet with several variations from Spy0128.

Gram-positive bacterial pilin proteins show wide variations in size and sequence, making it difficult to predict structural features based on sequence alone, and X-ray crystallography has played a critical role in elucidating structure and function of Gram-positive pili. There are now several other crystal structures are available, for both major and minor pilins, and these structures clearly point to common principles for many Gram-positive pilus assembly, in which self-generated intramolecular isopeptide bonds complement the sortase-mediated intersubunit bonds. These bonds are strategically located to give strength and stability to the pilin subunits and also facilitate proper assembly of pilus. Within this common theme, however, there are several variations that can account for their functional differences.

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**Keywords: bacterial pilus, isopeptide bond, assembly**

## MS.36.4

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**Structural investigations of a novel bacterial $AB_5$ toxin**

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$AB_5$ toxins are important virulence factors for several major bacterial pathogens including *Bordetella pertussis*, *Vibrio cholerae*, *Shigella dysenteriae* and at least two distinct pathotypes of *Escherichia coli* [1]. They are responsible for massive global morbidity and mortality, accounting for millions of deaths each year, particularly amongst children in developing countries. These toxins are so termed because they comprise a catalytic A-subunit (ADP-ribosylase or RNA N-glycosidase or subtilase activity) that is responsible for toxicity to the host cell, and that is non-covalently linked to a pentameric B- subunit that binds to glycans of the host cell [2]. We separately determined the crystal structures of the A- and B-subunits of a novel $AB_5$ toxin (SubAB) secreted by Shiga toxigenic *Escherichia coli* (STEC) to 1.8 Å [3] and 2.08 Å [4], respectively. The study revealed that the A-subunit of this toxin exhibited not only a novel catalytic activity (Subtilase) by targeting the ER chaperone BiP but also showed the first example of a bacterial toxin presenting a marked preference for a non-human synthesised Neu5Gc-containing glycans. We also structurally investigated further the glycan specificity of the B-subunit and these findings will be discussed. More recently, the X-ray structure of the SubAB holotoxin was determined to 2.6 Å. Alanine site directed mutagenesis of the B-subunit amino acid residues that are structurally contacting residues in the A-subunit was performed in order to obtain structural insights into the assembly of such unique architecture. These latest results will be discussed.

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**Keywords: AB5, toxin, glycans**

## MS.36.5

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**Toxicity and neutralization mechanism of the *mqsRA* toxin: antitoxin module**

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One mechanism by which bacteria survive environmental stress is through the formation of bacterial persister cells, a subpopulation of genetically identical quiescent cells that exhibit multidrug tolerance and are highly enriched in bacterial toxins. Persister cells are also capable of regenerating a bacterial population after removal of antibiotic, which poses considerable therapeutic challenges given that biofilms are present in over 85% of bacterial infections. Recently, it has been shown that a class of proteins, known as toxin:antitoxin (TA) modules, is highly upregulated in persister cells. Under normal conditions, the toxin and antitoxin associate to form a tight, non-toxic complex. However, under conditions of stress, the antitoxins are readily degraded and the action of the toxins leads to rapid cell growth arrest. The most highly upregulated gene in *Escherichia coli* persister cells is the bacterial toxin *mqsR* [1].

Here we used genetic, biochemical and structural studies to show that MqsR, along with MqsA, are a *bona fide* TA pair that define an entirely novel family of TA modules. The crystal structures (MqsA antitoxin alone, 2.15 Å; the MqsR:MqsA-N complex, 2.0 Å and the MqsA:DNA complex, 2.1 Å [2, 3]) show that the MqsR toxin is a ribonuclease from the RelE bacterial toxin family. MqsR is unique because it is the first toxin linked to biofilms and quorum sensing and is the first toxin that, when deleted, decreases persister cell formation. The antitoxin MqsA is even more atypical because it is the first antitoxin that requires a metal, zinc, for structural stability; it is the only *E. coli* antitoxin that is structured throughout its entire sequence; and it is the first antitoxin demonstrated to bind DNA via its C-terminal and not N-terminal domain. Critically, MqsA and the MqsR:MqsA complex are also the only known antitoxin/TA pair that bind not only their own promoter but also the promoters of other genes that play important roles in *E. coli* physiology, including *mcbR, spy, cspD* and the master regulator of stress, *rpoS* [4]. In addition, our structures also show that upon DNA binding, the MqsA N-terminal domains rotate more than 100° to 'clamp' the bound DNA. The binding interaction also induces a 55° curvature of the DNA duplex. Using EMSA and NMR titration experiments, we show that MqsA uses a mechanism of direct readout, mediated by residues Asn97 and Arg101, for DNA recognition. Finally, our most recent work is providing novel insights into the mechanism of MqsR-mediated mRNA cleavage. Collectively, these studies provide the first insights into understanding how the unique MqsR:MqsA TA system mediates the persister phenotype at a molecular level, and, by extension, the multidrug tolerance of *E. coli* biofilms, which are now being used to develop novel antibacterial therapies that target TA pairs.

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## MS.37.1

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**Potential impact of an X-FEL on time-resolved studies of protein dynamics**

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Structural biology is a very successful sub-field of the life-sciences. Technical innovations, including constant improvements surrounding the use of synchrotron radiation, have contributed to an extended acceleration in the rate at which new structures are determined.