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**Keywords: cytokine, receptor, structure**

## MS.36.2

*Acta Cryst.* (2011) A**67,** C90

**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

*Acta Cryst.* (2011) A**67,** C90

**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

*Acta Cryst.* (2011) A**67,** C90-C91

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

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