like a tetramer. Ultracentrifugation and native PAGE results also do not conclusively show whether it is a trimer or tetramer [4]. Initial crystallographic data however showed 3 molecules in the asymmetric unit suggesting an elongated trimer. SAXS scattering curve of the IgD binding domain is indicative of a trimeric arrangement and *ab initio* modeling confirms fibrous elongated shape. Recently circular dichroism spectroscopy was done on MID962-1200 and the data were deconvoluted using DICHROWEB [5]. The analyzed data with good NRMSD value shows approximately 11% alpha helices, 32% beta sheets and 30% unordered secondary structure.

We crystallized the protein MID962-1200 with His-tag at concentration 10 mg/ml, but had problem with reproducibility limiting our ability for experimental phasing.Now we have recloned it in pETM-30 with a cleavable GST-tag, which is digested by TEV protease [6] made in house, to allow for crystallization of the protein without any tag. The crystal structure of MID962-1200 domain alone and in complex with its partners like IgD will elucidate the mechanism of this protein and give information on host-pathogen interactions.

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Keywords: autotransporter, elongated, dichroism

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Elucidating the functions of Key regulators in biofilm formation and dispersal

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Biofilms are complex communities of bacteria that are encased in an extracellular matrix and adhere to almost any surface. They are also responsible for more than 65–80% of human infections. Moreover, these infections are extremely difficult to treat because biofilms are both highly resistant to host defenses and antibiotics. Currently, a detailed understanding of how biofilms assemble, how they are regulated at a molecular level, and how they achieve antibiotic resistance is only rudimentarily understood. Recent microarray studies have identified many of the genes that are up and down regulated in *E. coli* biofilm formation. We are using X-ray crystallography, combined with genetic and biochemical experiments, to determine the function of these proteins in order to understand their roles in biofilm formation and stability. Here, we report the expression, purification, crystallization and structures of two of these biofilm proteins, one which mediates biofilm dispersal (2.0 Å) and a second which directs biofilm formation (2.8 Å). Complementary genetic and biochemical experiments (electrophoretic mobility shift assays and isothermal thermal calorimetry) using the structural information as a guide are now being used to elucidate their *in vivo* ligands and functions. These studies are providing novel insights into the protein products that drive biofilm formation, dispersal and stability which, in turn, can be used as targets for the development of novel drugs to treat biofilms in the environment and disease.

Keywords: biofilm, E. coli

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Crystal structure of *S.aureus* AtlE homologous to the glucosaminidase domain of major AtlA

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Autolysins are a diverse group of enzymes responsible for degradation of peptidoglycans forming the bacterial cell wall. They are involved in a number of cellular processes including the cell wall expansion and cell division. They are also implicated in the bacterial pathogenesis. It was shown that autolysin deficient mutants of many bacterial strains exhibit lower virulence than their parental wild-type strains.

Methicillin-resistant strain of *Staphylococcus aureus* (MRSA) is a multidrug-resistant bacterium responsible for several difficult-to-treat infections in humans. The major autolysin A (AtlA) is the predominant autolysin in *Staphylococcus aureus*. It consists of N-terminal aminidase domain followed by the three cell-wall binding repeats and the C-terminal glucosaminidase domain. The genome of *Staphylococcus aureus*, however, contains additional autolysins. Here we present the crystal structure of Staphylococcus aureus autolysin E (AtlE) which exhibits high similarity to the glucosaminidase domain of AtlA. AtlE adopts a heart like fold. Despite no amino acid sequence homology between the AtlE and lysosome, the central helical core of AtlE alignes to the core structure of lysozyme. The two structurally unique subdomains of AtlE located at the top left and right side of the core domain additionally expand the structure. At the interface of both domains a deep and extended active site cleft is formed with a number of conserved Asp and Glu residues.

Functional characterisation of AtlE showed that the enzyme exhibits cell wall degrading activity and stimulates the formation and growth of biofilms. Since many infections caused by Staphylococcus aureus appear to be associated with biofilms, the AtlE structure may assist in the development of novel antibiotics.

Keywords: autolysin, glucosaminidase, biofilm

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Acoustically mounted microcrystals yield high resolution X-Ray structures

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Microcrystals measuring only a few microns along an edge are often easy to obtain but difficult to use because they are too small to yield a suitable diffraction pattern with conventional macromolecular crystallography (MX). Fortunately, advances in X-ray sources at third generation synchrotrons and free electron lasers (FEL) are rapidly reducing the sample size and exposure time required for atomic level crystal structure determination. However, as the crystal size is reduced, so is the signal relative to the noise in the X-ray diffraction data. Consequently, an essential strategy to improve the signal to noise ratio is to reduce the background scattering, especially from the mother liquor surrounding a micron-sized crystal. Robust new strategies must be developed to manipulate microcrystals for structure determination.

To address this critical gap, we are developing acoustic droplet