enzyme activity, oligomerisation state, interaction with other proteins, sub-cellular localization or half-life. Signal transduction through reversible protein phosphorylation is a key regulatory mechanism of both prokaryotes and eukaryotes. Phosphorylation frequently occurs in response to environmental signals and is mediated by specific protein kinases.

Recent studies reported that the eukaryotic-type serine/threonine kinase PrkC from *Bacillus subtilis* is also involved in bacterial exit from dormancy [1]. Under conditions of nutritional limitation, *B. subtilis* produces dormant spores, which are resistant to harsh environmental conditions and can survive in a dormant state for years [1].

Generally, growing bacteria release muropeptides in the surrounding environment, due to cell wall peptidoglycan remodelling associated to cell growth and division [1-7]. Therefore, the presence of muropeptides in the close environment of dormant bacteria is a clear signal that conditions are optimal for growth. The process of bacterial cell growth and resuscitation regulation in pathogenic bacteria will be discussed.

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Keywords: bacterial, pathogen, protein structure.

MS36.P28

Acta Cryst. (2011) A**67,** C479

Crystallization and preliminary X-ray diffraction analysis of a thioredoxin from *Streptococcus pneumoniae*

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Thioredoxins (TRX) are ubiquitous proteins involved on a wide number of critical celular functions comprising protein folding and repair, DNA synthesis and oxidative stress response [1]. These proteins share a conserved active sequence site [Cys-X-X-Cys] and a common 3D architecture known as thioredoxin motif composed of four α-helices and five β-sheets. TRX are responsible of keeping the cellular reducing environment accepting electrons from a donor by NADPH reduction and transferring them to other acceptors. Recently, a pneumoccocal thioredoxine-like protein have been crystalize using the hanging-drop vapour-diffusion method at 291K. Diffraction quality of tetragonal crystals belongs to space group $P4_32_12$ with unit-cell parameters a = 62.85*, b* = 62.85 *and c* = 89.60Å. X-ray data sets were obtained up to 1.3Å. structural characterization and functional properties are currently undergoing.

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Keywords: thioredoxin, macromolecules, redox protein

MS36.P29

Acta Cryst. (2011) A**67,** C479

Crystal structure of the class D β-lactamase OXA-40

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β−Lactam antibiotics have been widely used since World War II. These antibiotics efficiently inhibit bacterial peptidoglycan trans-peptidases, which leads to cell lysis and death of the growing bacteria. β-Lactamases hydrolyze the β-lactam ring of β-lactam antibiotics following an acylation and deacylation steps. β-Lactamases are divided into four classes, A, B, C and D, according to their sequence similarities. Class B enzymes are metalloproteins that require a zinc ion for their enzymatic activity, whereas classes A, C and D β-lactamases contain serine residue in their active site.

OXA-40 is a class D β-lactamase isolated from *Acinetobacter baumannii.* OXA-40 hydrolyzes carbapenems which are one of the antibiotics of last resort for many bacterial infections. In this study we expressed OXA-40 in *E. coli* and purified. Crystals suitable for X-ray structure determination were obtained by hanging drop vapor diffusion method at pH 8.5. The crystals belong to space group $P4_12_12$ with cell dimensions a=b=102.6 Å, c=84.9Å. The three dimensional structure of OXA-40 has been solved by the molecular replacement method using OXA-24 as a search model and refined to 1.53 Å resolution.

OXA-40 is a monomeric enzyme which consists of two domains, one containing five α-helices and the other one containing a six-stranded antiparallel β-sheet flanked by N- and C-terminal helices on one side, and a helix on the other side. The active site lies at the junction of the two domains. The general base Lys84 in the active site is carbamylated. Crystallization conditions of complexes with β-lactam antibiotics are being searched.

Keywords: β-lactamase, carbapenem resistance, X-ray analysis

MS36.P30

Acta Cryst. (2011) A**67,** C479-C480

Crystallization of MID962-1200: A trimeric autotransporter from *M. catarrhalis*

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Moraxella catarrhalis is a newly emerging pathogenic bacterium that is involved in otitis media and sinusitis in children as well as lower respiratory tract infections in adults with chronic obstructive pulmonary disease (COPD). Over the last 20 to 30 years, the bacterium has emerged as a genuine pathogen. In immuno compromised hosts, the bacterium can cause a variety of severe infections including pneumonia, endocarditis, septicemia, and meningitis [1]. Today more than 90% of all clinical isolates are β-lactam resistant [2]. One of the most important virulence factors for *M. catarrhalis* is a 200 kDa outer membrane protein, which is responsible for the IgD binding, namely the Moraxella IgD-binding (MID) protein [3]. MID belongs to the trimeric autotransporter protein family and has N-terminal signal peptide, internal passenger domain and C-terminal translocator domain.

Having an elongated shape the trimeric MID962-1200 shows misleading results in size exclusion chromatography and behaves

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

MS36.P31

Acta Cryst. (2011) A**67,** C480

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

MS36.P32

Acta Cryst. (2011) A**67,** C480

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

MS37.P01

Acta Cryst. (2011) A**67,** C480-C481

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