functional and structural studies were performed, focusing on SmeDEF, the most relevant antibiotic- and triclosan-removing multidrug efflux pump of *S. maltophilia*. Expression of *smeDEF* is regulated by the repressor SmeT. Triclosan released SmeT from its operator and induces the expression of *smeDEF*, thus reducing the susceptibility of *S. maltophilia* to antibiotics in the presence of the biocide. The structure of SmeT bound to triclosan is described. Two molecules of triclosan were found to bind to one subunit of the SmeT homodimer. The binding of the biocide stabilizes the N terminal domain of both subunits in a conformation unable to bind DNA. This complex structure is the first structural evidence of the ability of triclosan to act as an effector via its binding to a transcriptional regulator (SmeT). Given that SmeT mediate the susceptibility of *Stenotrophomonas maltophilia* to antibiotics by repressing SmeDEF expression, the present results provide information that aids our understanding of the molecular basis of biocide-induced antibiotic resistance.

Keywords: complex, ligand, antibacterial

MS36.P25

Acta Cryst. (2011) A**67,** C478

Interaction of the type III secretion chaperone SycD with YscY

Madeleine Schreiner,, Hartmut Niemann, *Department of Chemistry, Bielefeld University, Bielefeld (Germany).* E-mail: madeleine.schreiner@uni-bielefeld.de

The type III secretion system (T3SS) is used by several Gram-negative pathogenic bacteria to inject cytotoxins, so called effector proteins, into the host cell to manipulate their host for their own benefit. The effector translocation occurs via a needle-like nanomachine, the injectisome, which spans the whole bacterial envelope. The effectors enter the cell through a pore within the host cell membrane formed by bacterial translocator proteins. Both the effectors and translocators need chaperones for their efficient translocation. T3S chaperones work without the need for ATP hydrolysis and are divided into three subclasses: class I chaperones interacting with effector proteins, class II chaperones interacting with translocator proteins and class III chaperones interacting with needle components.[1]

SycD (specific yop chaperone D) is the class II chaperone of the translocators YopB (*Yersinia* outer protein B) and YopD from the enteropathogen *Yersinia enterocolitica*. Additionally, SycD plays an important role in diverse regulatory processes of the T3SS and interacts with several other T3S proteins like TyeA, YscM2 or YscY. Like all structurally characterised class II chaperones SycD comprises an overall α-helical fold consisting of three tetratricopeptide repeats (TPR) providing a concave hydrophobic groove for translocator binding. SycD is known to form homodimers in solution involving the residues A61 and L65 of the first TPR as binding platform and mutations within these dimerisation-mediating residues lead to a stable monomeric chaperone that is not able to rescue a *sycD* null mutant of *Y. enterocolitica*.[2] Thus the dimerisation is either essential for the chaperone function or the TPR1 provides a further binding region for other T3S proteins interacting with SycD.

In order to further characterise the interaction of SycD with its various binding partners we coexpressed and copurified SycD together with YscY, a 114 aa protein which is proposed to be the chaperone of the secreted T3S protein YscX (*Yersinia* secretion X). We confirmed that both proteins interact via the N-terminal region of SycD involving the first TPR1 forming an elongated heterodimeric 1:1 complex which is preferred over SycD dimerisation. Furthermore, the monomeric SycD variant containing mutations at the positions A61 and L65 within the first TPR is not able to bind YscY. Hence the SycD/YscY complex formation comprises the same binding region that is also involved in SycD dimerisation. One might conclude, that not the SycD dimerisation itself is essential for chaperone function within the T3SS, but the formation of heterodimeric or even larger multi-protein complexes.

[1] J.E. Galán, H. Wolf-Watz, *Nature* **2006**, *444*, 567-573. [2] C.R. Büttner, I. Sorg, G.R. Cornelis, D.W. Heinz, H.H. Niemann, *J. Mol. Biol.* **2008**, *375*, 997-1012.

Keywords: chaperone, interaction_interface, protein_complex

MS36.P26

Acta Cryst. (2011) A**67,** C478

Cell wall modelling in pathogenic bacteria

Alessia Ruggiero,[a,b] Flavia Squeglia,[a,b] Luciano Pirone,[a] Stefania Correale,[a] Rita Berisio,[a] [a]*Institute of Biostructure and Bioimaging, via Mezzocannone 16, I-80134 Napoli, (Italy).* [b]*University Federico II of Naples, via Cinthia, I-80126 Naples, (Italy).* E-mail: alessia.ruggiero@unina.it

The interaction between bacterial pathogens and the human host after infection may manifest itself as a chronic disease or as a latent (or dormant) infection, a state capable to evade host responses. The probability of reactivation from dormancy is strongly affected by the type of host immune response and it is significantly enhanced in immuno-compromised patients, e.g. suffering from AIDS. Understanding and controlling the entry and exit from dormancy is important in the development of new anti-microbial therapies.

Resuscitation of dormant bacteria is promoted by a set of peptidoglycan hydrolases, which are secreted from slowly replicating bacteria in the extra-cellular milieu. By cleaving peptidoglycans which constitute the cell wall, these hydrolases are thought to alter cell wall mechanical properties and favour cell division and/or release anti-dormancy factors. We have determined the crystal structures of various cell wall modelling enzymes [1-5]. The comprehension of the structural features associated to cell wall modelling enzymes activity/inhibition will provide the bases for the identification of molecules (pro-latency molecules) able to restrict bacterial life to the latent, non-dangerous, state.

[1] A. Ruggiero, B. Tizzano, A. Geerlof, E. Pedone, C. Pedone, M. Wilmanns, R. Berisio *Acta Crystallogr Sect F* **2007,** *63*, 870-873. [2] A. Ruggiero, B. Tizzano, E. Pedone, C. Pedone, M. Wilmanns, R. Berisio, *J. Mol. Biol.* **2009**, *385,* 153-62. [3] A. Ruggiero, D. Marasco, F. Squeglia, S. Soldini, E. Pedone, C. Pedone, R. Berisio, *Structure* **2010**, *18*, 1184-1190. [4] A. Ruggiero, F. Squeglia, C. Esposito, D. Marasco, E. Pedone, C. Pedone, R. Berisio, *Protein Pept Lett*. **2010**, *17*, 70-73. [5] A. Ruggiero, F. Squeglia, L. Pirone, S. Correale, R. Berisio, *Acta Crystallogr Sect F* **2011,** *67*, 164-168.

Keywords: latency, bacteria, structure.

MS36.P27

Acta Cryst. (2011) A**67,** C478-C479

Muropeptide-driven revival from dormancy in bacterial pathogens: a structural perspective

Flavia Squeglia,[a,b] Alessia Ruggiero,[a,b] Daniela Marasco,[b] Rita Berisio,[a] [a]*Institute of Biostructure and Bioimaging, via Mezzocannone 16, I-80134 Napoli, (Italy).* [b]*University Federico II of Naples, via Cinthia, I-80126 Naples, (Italy).*

Post-translational modifications are a ubiquitous means of rapidly and reversibly modifying the physical-chemical properties of a protein, triggering a number of possible consequences: change of

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.

[1] I. Shah, M. Laaberki, M.H. Popham, J. Dworkin, *Cell* **2008,** *135*, 486-96. [2] A. Ruggiero, B. Tizzano, E. Pedone, C. Pedone, M. Wilmanns, R. Berisio, *J. Mol. Biol.* **2009**, *385*, 153-62. [3] A. Ruggiero, D. Marasco, F. Squeglia, S. Soldini, E. Pedone, C. Pedone, R. Berisio, *Structure* **2010**, *18,* 1184-1190. [4] A. Ruggiero, F. Squeglia, C. Esposito, D. Marasco, E. Pedone, C. Pedone, R. Berisio, *Protein Pept Lett*. **2010**, *17*, 70-73. [5] A. Ruggiero, F. Squeglia, L. Pirone, S. Correale, R. Berisio, *Acta Crystallogr Sect F* **2011,** *67*, 164-168. [6] A. Ruggiero, F. Squeglia, V. Izzo, A. Silipo, L. Vitagliano, A. Molinaro, R. Berisio, *Protein Pept Lett*. **2010**, *17*, 1296-1299. [7] A. Ruggiero, F. Squeglia, D. Marasco, R. Marchetti, A. Molinaro, R. Berisio, *Biochem J*. **2011**, *435,* 33-41.

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*

Sergio Galán Bartual,[a] Malek Saleh,[b] Sven Hammerschmidt,[b] and Juan Antonio Hermoso[a] [a]*Department of Crystallography and Structural Biology, Instituto de Química Física "Rocasolano", CSIC, Madrid.* [b]*Institute for Genetics and Functional Genomics, Department of Genetics of Microorganisms, Greifswald, (Germany).* E-mail: xsergio@iqfr.csic.es

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.

[1] T. Zeller, G. Klug, *Naturwissenschaften* **2006,** *93*, 259-266.

Keywords: thioredoxin, macromolecules, redox protein

MS36.P29

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

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

Akira Uchida,[a] Yoshikazu Ishii,[b] Yuichi Kouyama,[b] Kazuhiro Tateda,[b] [a]*Department of Biomolecular Science, Faculty of Science, Toho University, Chiba 274-8510, (Japan).* [b]*Department of Microbiology and Infectious Diseases, Faculty of Medicine, Toho University, Tokyo 143-8540, (Japan).* E-mail: auchida@biomol.sci.toho-u.ac.jp

. β−Lactam antibiotics have been widely used since World War II. These antibiotics efficiently inhibit bacterial peptidoglycan transpeptidases, 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*

Mahmudul Hasan,[a] Karolina Förnvik,[a] Marjolein Thunnissen,[a] and Kristian Riesbeck,[b] [a]*Department of Biochemistry and Structural Biology, Kemicentrum, Lund University, Lund, (Sweden).* [b]*Medical Microbiology, Department of Laboratory Medicine, Lund University, Malmö University Hospital, Malmö, (Sweden).* Email: mahmudul.hasan@biochemistry.lu.se

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