

Poster Sessions

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

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Interaction of the type III secretion chaperone SycD with YscY
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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.

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Cell wall modelling in pathogenic bacteria

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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.

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

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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