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AB_5 toxins are important virulence factors for several major bacterial pathogens including *Bordetella pertussis*, *Vibrio cholerae*, *Shigella dysenteriae* and at least two distinct pathotypes of *Escherichia coli* [1]. They are responsible for massive global morbidity and mortality, accounting for millions of deaths each year, particularly amongst children in developing countries. These toxins are so termed because they comprise a catalytic A-subunit (ADP-ribosylase or RNA N-glycosidase or subtilase activity) that is responsible for toxicity to the host cell, and that is non-covalently linked to a pentameric B- subunit that binds to glycans of the host cell [2]. We separately determined the crystal structures of the A- and B-subunits of a novel AB_5 toxin (SubAB) secreted by Shiga toxigenic *Escherichia coli* (STEC) to 1.8 Å [3] and 2.08 Å [4], respectively. The study revealed that the A-subunit of this toxin exhibited not only a novel catalytic activity (Subtilase) by targeting the ER chaperone BiP but also showed the first example of a bacterial toxin presenting a marked preference for a non-human synthesised Neu5Gc-containing glycans. We also structurally investigated further the glycan specificity of the B-subunit and these findings will be discussed. More recently, the X-ray structure of the SubAB holotoxin was determined to 2.6 Å. Alanine site directed mutagenesis of the B-subunit amino acid residues that are structurally contacting residues in the A-subunit was performed in order to obtain structural insights into the assembly of such unique architecture. These latest results will be discussed.

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Toxicity and neutralization mechanism of the *mqsRA* toxin: antitoxin module

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One mechanism by which bacteria survive environmental stress is through the formation of bacterial persister cells, a subpopulation of genetically identical quiescent cells that exhibit multidrug tolerance and are highly enriched in bacterial toxins. Persister cells are also capable of regenerating a bacterial population after removal of antibiotic, which poses considerable therapeutic challenges given that biofilms are present in over 85% of bacterial infections. Recently, it has been shown that a class of proteins, known as toxin:antitoxin (TA) modules, is highly upregulated in persister cells. Under normal conditions, the toxin and antitoxin associate to form a tight, non-toxic complex. However, under conditions of stress, the antitoxins are readily degraded and the action of the toxins leads to rapid cell growth arrest. The most highly upregulated gene in *Escherichia coli* persister cells is the bacterial toxin *mqsR* [1].

Here we used genetic, biochemical and structural studies to show that MqsR, along with MqsA, are a *bona fide* TA pair that define an entirely novel family of TA modules. The crystal structures (MqsA antitoxin alone, 2.15 Å; the MqsR:MqsA-N complex, 2.0 Å and the MqsA:DNA complex, 2.1 Å [2, 3]) show that the MqsR toxin is a ribonuclease from the RelE bacterial toxin family. MqsR is unique because it is the first toxin linked to biofilms and quorum sensing and is the first toxin that, when deleted, decreases persister cell formation. The antitoxin MqsA is even more atypical because it is the first antitoxin that requires a metal, zinc, for structural stability; it is the only *E. coli* antitoxin that is structured throughout its entire sequence; and it is the first antitoxin demonstrated to bind DNA via its C-terminal and not N-terminal domain. Critically, MqsA and the MqsR:MqsA complex are also the only known antitoxin/TA pair that bind not only their own promoter but also the promoters of other genes that play important roles in *E. coli* physiology, including *mcbR, spy, cspD* and the master regulator of stress, *rpoS* [4]. In addition, our structures also show that upon DNA binding, the MqsA N-terminal domains rotate more than 100° to 'clamp' the bound DNA. The binding interaction also induces a 55° curvature of the DNA duplex. Using EMSA and NMR titration experiments, we show that MqsA uses a mechanism of direct readout, mediated by residues Asn97 and Arg101, for DNA recognition. Finally, our most recent work is providing novel insights into the mechanism of MqsR-mediated mRNA cleavage. Collectively, these studies provide the first insights into understanding how the unique MqsR:MqsA TA system mediates the persister phenotype at a molecular level, and, by extension, the multidrug tolerance of *E. coli* biofilms, which are now being used to develop novel antibacterial therapies that target TA pairs.

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Potential impact of an X-FEL on time-resolved studies of protein dynamics

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Structural biology is a very successful sub-field of the life-sciences. Technical innovations, including constant improvements surrounding the use of synchrotron radiation, have contributed to an extended acceleration in the rate at which new structures are determined.