	Experiment title: Structural of SatA – a xenobiotic acetyl transferase from Enterococcus faecium implicated in post-operative antibiotuic resistance	**Experiment number**: LS 1778
Beamline: BM 14	**Date of experiment**: from: 8/11/00 to: 10/11/00	**Date of report**: July 2003
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Report:

Structural basis of synercid (quinupristin-dalfopristin) resistance in gram-positive bacterial pathogens

J.Biol.Chem. 2003 in press.

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Abstract

Synercid, a new semisynthetic streptogramin-derived antibiotic containing dalfopristin and quinupristin, is used in the treatment of life-threatening infections caused by glycopeptide resistant Enterococcus faecium and other multidrug resistant gram-positive bacterial pathogens. However, dissemination of genes encoding virginiamycin acetyltransferases, homotrimeric enzymes that confer resistance to streptogramins, threatens to limit the medical utility of the quinupristin-dalfopristin combination. Here we present structures of virginiamycin acetyltransferase D (VatD) determined at 1.8 Å resolution in the absence of ligands, at 2.8 Å resolution bound to dalfopristin and at 3.0 Å resolution in the presence of the second substrate acetyl-coenzyme A (AcCoA). Our structural data reveal that dalfopristin is bound by VatD in a similar conformation to that previously described for the A-class streptogramin virginiamycin M1. However, specific interactions with the substrate are altered as a consequence of a conformational change in the pyrollidine ring that is propagated to adjacent constituents of the dalfopristin macrocycle. Inactivation of dalfopristin involves acetyl transfer from AcCoA to the sole (O18) hydroxyl group of the antibiotic. In the binary complex this hydroxyl group lies close to the sidechain of the strictly conserved residue, His-82. Replacement of residue 82 by alanine via site-directed mutagenesis is accompanied by a fall in specific

activity of >105-fold, indicating that the imidazole moiety of His-82 is a major determinant of catalytic rate enhancement by VatD. The structure of the VatD-dalfopristin binary complex can also be used to predict positions where further structural modification of the drug might preclude enzyme binding and thereby circumvent Synercid resistance conferred by virginiamycin acetyltransferases.