[MS5-P30] Structure and Functional Study of tRNA Dependent Fem Ligases in *Staphylococcus aureus*. Konstantin A. Fritz[1], Jennifer Shephard[1], Adrian J. Lloyd[1], David I. Roper[1],

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Staphylococcus aureus is a well characterised Gram-positive human pathogen which can cause septicaemia and abscesses in several organs and is a significant factor in both community and hospital acquired infections.[1] Extracellular cross-linking of mature peptidoglycan is dependent on the presence of pentaglycine interpeptide bridges.[2] The pentaglycyl extensions between adjacent stem peptides are dependent upon the enzymatic activity of factors essential for methicillin resistance proteins (FemX, FemA & FemB). These act by sequentially adding glycine residues at the inner face of the cytoplasmic membrane using glycyl- tRNA cofactors. Although the crystal structure of FemA has been determined [3] little is known about the exact mechanism of any Fem proteins' catalytic activity, especially with regards to their substrate specificity and binding. FemA and FemB have been expressed using BL21(DE3)Star.pRosetta cells, and purified using cobalt based immobilised metal affinity chromatography. Crystallisation conditions have been identified for both FemA and FemB using the JCSG plus HT-96 and PACT premier HT-96 screens (Molecular Dimensions). Suitable crystallisation conditions for FemX will be investigated. Data for FemA has been collected to 1.9 angstrom resolution. Future work will include the reconstitution of glycyl-tRNA dependent activity of each Fem protein with the various glycyl-tRNA species found in *S. aureus* and their linkage to penicillin binding protein activity.

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