

MS06-P05 | MOLECULAR BASIS OF SELF-INDUCED GENOMIC DEGRADATION INHIBITION BY THE SUB-NANOMOLAR INTERACTION OF NIN WITH NUCA AND NUCB ENDONUCLEASES

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Biofilms are communities of sessile bacteria that form on a wide variety of natural and man-made surfaces, sometimes at a detriment to human health. Bacteria in biofilms are held together by an extracellular matrix of polysaccharides, lipids, proteins, and extracellular DNA (eDNA). Species of *Bacillus* secrete two closely related endonucleases, Nuclease A and B (NucA & NucB), into their environment to degrade eDNA to ease its uptake by the cell either to enhance their genetic diversity, or for metabolic purposes, respectively. As a mechanism of protection from self-induced genome degradation, the genes for NucA and a putative cytosolic inhibitor, Nin, are present in a bi-cistronic operon presumably to drive co-expression.

Through a combination of biophysical/biochemical techniques, we probed the interaction between NucA/B and Nin from *Bacillus subtilis*. The structures of NucA/NucB in complex with Nin were solved by X-ray crystallography, revealing the mechanism of inhibition by Nin, and allowing for the calculated dismantling of the complexes by site-directed mutagenesis. We found that single alanine substitutions in Nin at the interface between the two proteins were insufficient to disrupt the interaction, however, reversing charges on Nin at electrostatic interfaces in conjunction with alanine mutations were sufficient to abrogate binding, whilst maintaining the overall fold of Nin. Genetic studies confirmed the importance of the interfacial residues, and this model system now permits future studies of how the Nin/Nuc complexes are disassembled at the membrane to permit secretion of the endonuclease whilst leaving the host genome intact.