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Towards Anti-Virulence Antimicrobials

Discovery and Development of Sortase A Inhibitors and Investigations of Bacterial Phenotypes

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Abstract

Antibiotic resistance is an emerging and serious threat to public health. Immediate actions are required to preserve current antibiotics while intensifying research efforts towards the development of new effective therapeutics. A novel approach to combat bacterial infections focusses on the inhibition of bacterial virulence to inhibit disease-causing properties rather than bacterial growth. In several Gram-positive bacteria, the bacterial enzyme sortase A (SrtA) is critical for an intact cell surface display of virulence-associated proteins. Inhibition of SrtA is, therefore, expected to greatly reduce bacterial virulence, serving as a potential therapeutic approach to treat Gram-positive infections. In order to fully exploit novel intervention strategies we need to further improve our understanding of bacterial virulence, persistence and stress responses.

Firstly, this thesis describes the discovery, synthesis and evaluation of inhibitors of SrtA. Secondly, the phenotypic characterization of bacteria using Fourier-transform infrared (FTIR) spectroscopy as well as time-of-flight secondary ion mass spectrometry (ToF-SIMS) is discussed.

A new class of SrtA inhibitors was identified by high-throughput screening of ~ 28500 small-molecule compounds. Synthetic modification of hit structures yielded a series of compounds that exhibited increased inhibitory activity in a functional, FRET based, assay. Ligand-detected protein binding experiments using Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion NMR spectroscopy confirmed binding to SrtA and guided the design of new structures. The reversibility of binding, binding kinetics, and binding affinity were determined by surface plasmon resonance (SPR) spectroscopy. All compounds tested displayed a reversible binding mode and some exhibited a very high binding affinity.

In a feasibility study, FTIR spectroscopy in combination with design of experiment and multivariate statistical analysis (MVA) was applied to explore the condition dependent phenotypic diversity of *Staphylococcus aureus*. Planktonic cultures of *S. aureus* were grown under various conditions according to the experimental design. FTIR spectra obtained from each treatment group contained distinct profiles that allowed full cluster separation in principal components analysis (PCA).

ToF-SIMS was employed for further and more detailed characterization of bacterial phenotypes by direct analysis of native cell samples. Initial experiments demonstrated the capability of ToF-SIMS, coupled with MVA, to fully differentiate *Escherichia coli*, *Pseudomonas aeruginosa*, as well as two strains of *S. aureus*. Further investigations focused more specifically on *E. coli* and explored the role of the stringent response in growth phase dependent lipid modifications. Mass spectral assignments revealed that a $ppGpp^0$ mutant exhibited alterations in lipid composition in stationary phase. Results suggest the occurrence of alternative stress response mechanisms that are regulated independently of ppGpp.

Keywords: Sortase A, SrtA, Inhibitors, Anti-virulence, Bacterial analysis, FTIR spectroscopy, Bacterial phenotyping, Design of Experiment, Multivariate Data Analysis, PCA, ToF-SIMS, Time-of-flight secondary-ion-mass-spectrometry, Lipid analysis, ppGpp, Stringent response.