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Developing autoinducing peptides for regulating the quorum sensing system in *S. lugdunensis*

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1 Background

Bacteria are highly adaptable microorganisms able to change their gene expression based on environmental changes such as pH or nutrient availability. Quorum sensing (QS) is a system used by many bacteria to sense the population density, and make corresponding changes in the gene expression pattern. This is done through a signaling molecule known as an autoinducing peptide (AIP). In *Staphylococcus aureus* the system causes an up-regulation in virulence related genes and a down-regulation of genes relevant for biofilm formation.[1]

In most staphylococcal bacteria, the QS system functions similarly to what is seen in *S. aureus*, however, cellular processes regulated by the quorum sensing system is different in different species. Substantial efforts have already been focused on developing inhibitors of the QS system in *S. aureus*, however, other species are under-represented in this regard. Our aim is therefore to synthesize an inhibitor of QS in *S. lugdunensis* to investigate the system further in this organism.

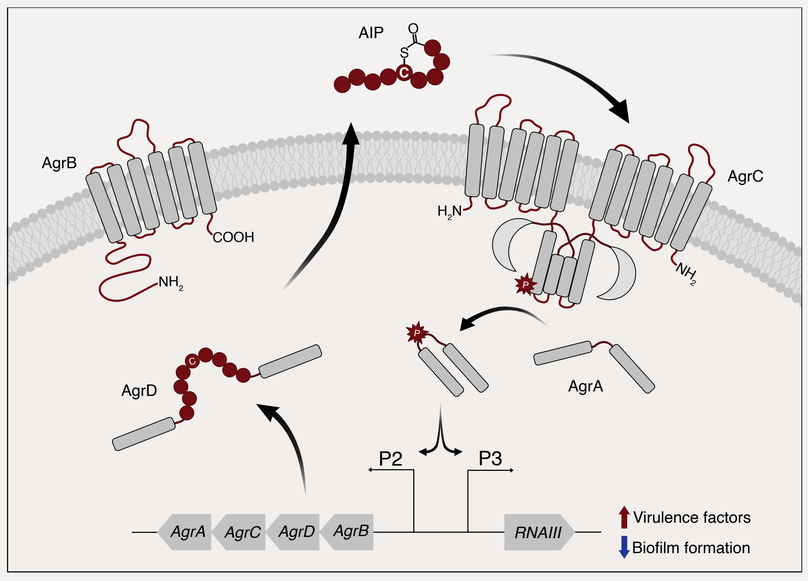


Figure 1: Schematic overview of the quorum sensing system in *S. aureus*. After translation of the accesory gene regulator (*agr*) locus, the precursor AgrD peptide is posttranslationally cleaved and cyclized into the final AIP. The AIP is then transported into the extracellular environment where it interacts with the receptor kinase AgrC. Activation of AgrC leads to phosphorylation of AgrA which can then bind to the P2 and P3 promoters, causing changes in gene expression.

2 QS cross-talk

Gless et al[2] have carried out extensive studies regarding the cross-inhibitory profile of a number of native AIPs from different species of *Staphylococcus*. The purpose of the study is to investigate how different species interfere with QS in case of co-habitation.

Testing the AIPs under the same conditions, makes it possible to directly compare the potency of different AIPs on the same reporter strain.

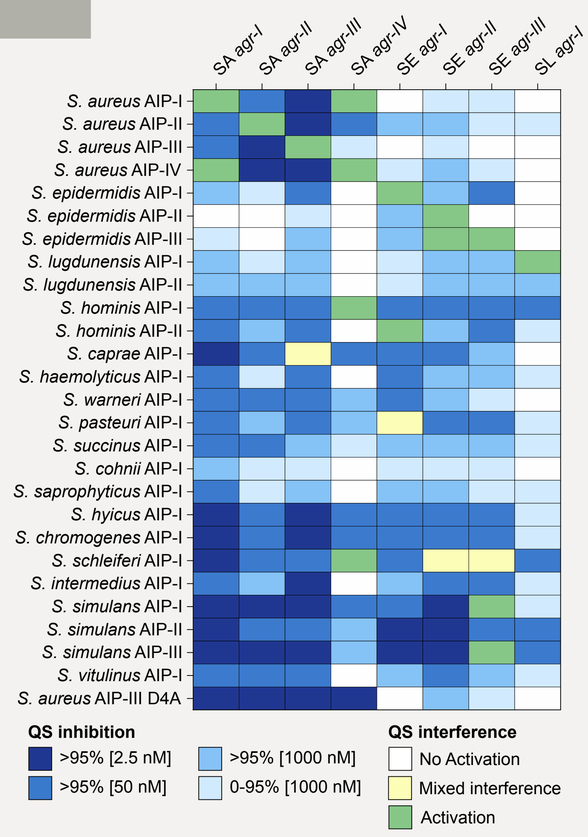


Figure 2: Synthetic AIPs were tested at multiple concentrations (1 µM, 50 nM, 2.5 nM) against eight fluorescent reporter strains of *S. aureus* (SA), *S. epidermidis* (SE), and *S. lugdunensis* (SL).[2]

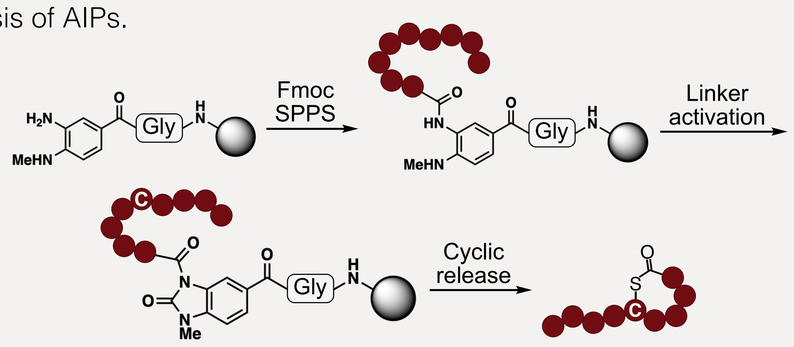
3 Sequence alignment

In the heatmap from Gless et al, we found that only a small number of native AIPs inhibit *S. lugdunensis* to a significant extent. These sequences were aligned to identify any common motif. The alignment showed high sequence similarity in the ring with a more variable tail region.

Figure 3: Alignment of peptide sequences from *S. lugdunensis* AIP-I, *S. hominis* AIP-II, *S. schleiferi* AIP-I, *S. simulans* AIP-II, and *S. simulans* AIP-III.

4 AIP synthesis

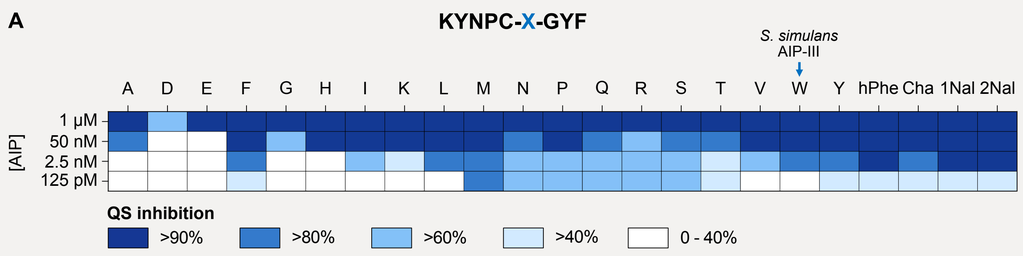
The characteristic thioester motif, found in most of the natural AIPs, can be installed by employing the chemistry introduced by Dawson and coworkers.[3] This chemistry was further optimized in our group by Gless et al[4] for simplified synthesis of AIPs.

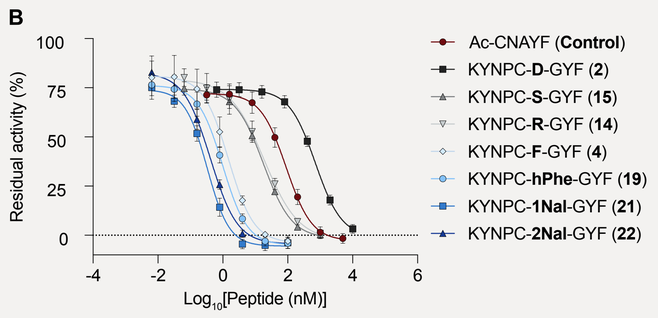


Scheme 1: The principle behind AIP synthesis using the MeDbz-linker.

5 Results

From the alignment of *S. lugdunensis* inhibiting AIPs, we decided to base our inhibitor series on the sequence KYNPC-**X**-GYF, where **X** represents a variable amino acid residue. Using this sequence, we investigated what functionalities were tolerated at position **X**. A representative selection of compounds, including the most potent, were then tested in a dose-response assay. As a control, we also tested an *S. lugdunensis* QS inhibitor reported previously.[5]



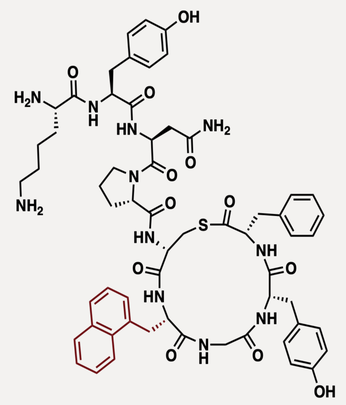


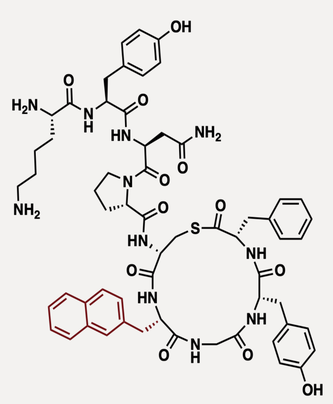
X	IC_{50}(nM)	CI(95%)
Trp	0.48[2]	
1Nal	0.33	0.27–0.40
2Nal	0.41	0.32–0.53

Figure 4: A) Inhibition data from 4-dose screening of initial compound series. B) Dose-response curves for selected compounds from the initial compound series.

6 Summary & outlook

- We designed a compound series to test against our *S. lugdunensis* fluorescent reporter strain.
- We found two lead candidates which showed potent inhibition.
- The next step is to test compounds **21** & **22** against our other reporter strains, and compare cross-interference with what is observed for *S. simulans* AIP-III.
- It would be interesting to then investigate what phenotype would result from *S. lugdunensis* QS inhibition, and if possible also activation.





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