Targeting Quorum Sensing in *Streptococcus pneumoniae*: An Alternative Antibacterial Approach

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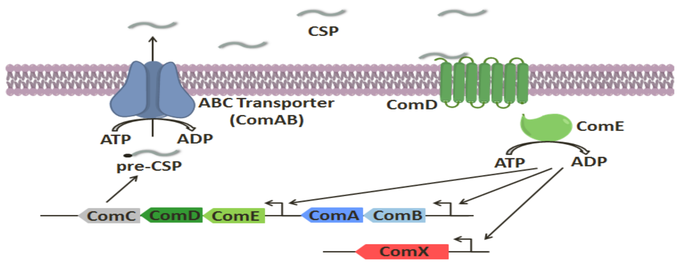
Introduction

Quorum sensing (QS) is a mechanism through which bacteria coordinate gene expression in response to cell density. QS is considered as an alternative antibacterial approach due to the potential to treat bacterial infections with minimal development of resistance. *Streptococcus pneumoniae* is an important pathogen that utilizes QS to regulate genetic transformation, virulence and biofilm formation. The competence stimulating peptide (CSP) is a 17-amino acid peptide signal that is used by *S. pneumoniae* to trigger QS. *S. pneumoniae* strains can be divided into two main specificity groups based on the CSP signal they produce (CSP1 or CSP2) and their compatible transmembrane histidine kinase receptor (ComD1 or ComD2 respectively). In our lab we aim to develop synthetic CSP-based analogs capable of modulating pneumococcal QS by intercepting the CSP:ComD interaction. Therefore, we performed full alanine and D-amino acid scans of CSP1 and tested the ability of the analogs to modulate QS in the two *S. pneumoniae* QS specificity groups. We identified several key residues that are critical to receptor binding, activation, and specificity, based on which we designed second-generation analogs. Additionally, we used CD spectroscopy to assess the global structural features of CSP1 and found that it adopts an α-helix conformation in membrane mimicking condition. We then evaluated the secondary structure of all the CSP1 analogs and identified a strong correlation between helicity and bioactivity. Moreover, our recent 2D-NMR studies on CSP1 and several analogues revealed an interesting amphiphilic feature of the helical structure that is likely required for effective ComD binding.

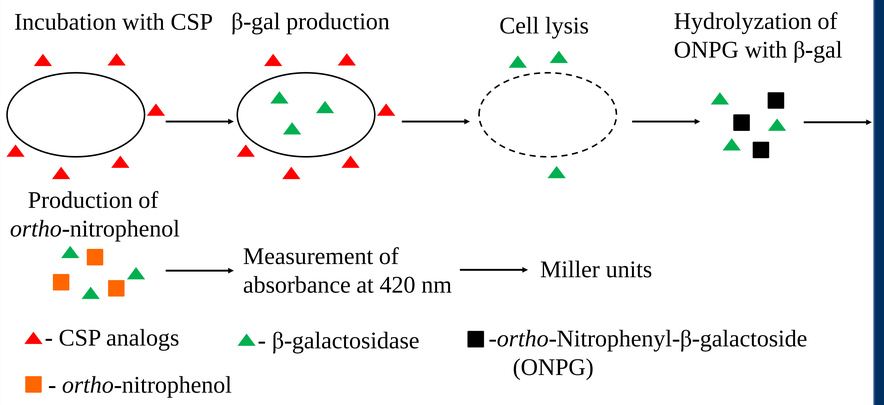
The CSP-Based QS Circuitry

CSP1 EMRLSKFFRDFILQRKK
CSP2 EMRISRIILDFLFLRKK

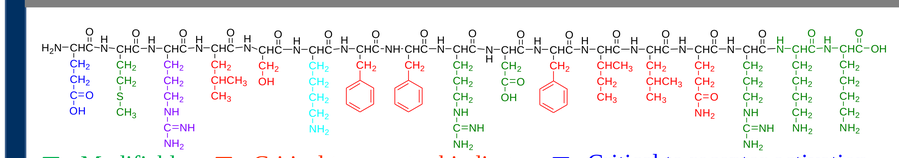


ComC gene encodes a pre-CSP peptide, which is processed and secreted by the ABC transporter (ComAB). As the culture grows, the concentration of CSP increases until it reaches a threshold. Then CSP activates a transmembrane histidine kinase receptor (ComD) which, after being activated, transfers a phosphate group to its cognate response regulator (ComE). After phosphorylation ComE triggers the transcription of numerous genes including *comX*, which is responsible for QS-regulated phenotypes.

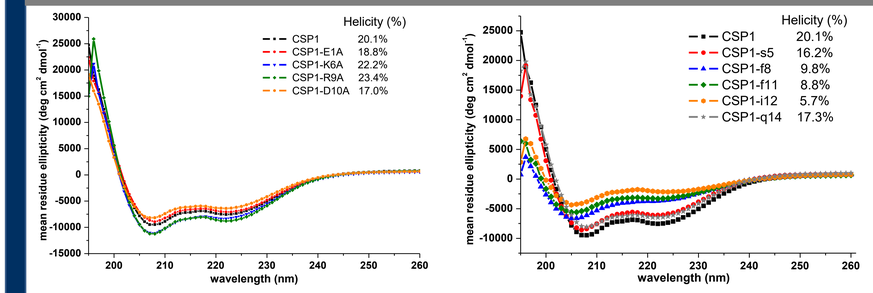
β-galactosidase Assay



Structure Activity Relationship of CSP1



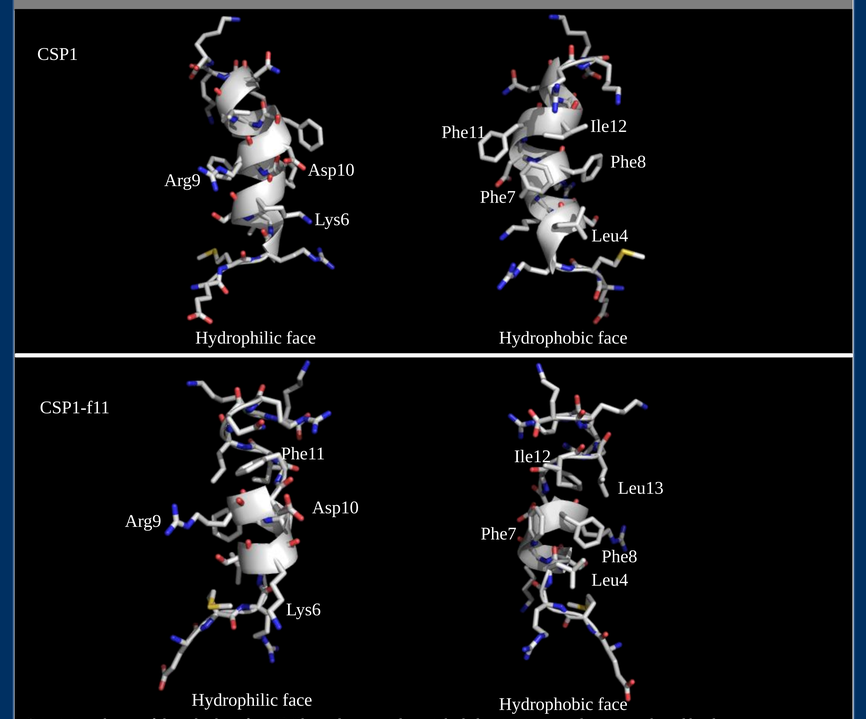
Alpha-Helix is Required for Effective Binding



Second-Generation CSP Analogs

Name	EC_{50} / IC_{50} (nM)	
	ComD1	ComD2
CSP1-des-K17	4.86	>1000
CSP1-des-K16K17	8.10	>1000
CSP1-E1AK6A	104	--

2D-NMR Analysis



Orientation change of the side chain from D-Phe replacement disrupt the helix structure, resulting in weakened binding.

Conclusions

- Key residues for receptor activation, receptor binding and specificity were identified in CSP1.
- Based on the structure activity relationship of CSP1, new analogs with novel activity profiles were designed.
- An alpha-helix conformation was found to be important to receptor binding.
- 2D-NMR analysis indicated that the hydrophobic cluster in the center of the helix was critical to receptor binding.

Acknowledgements

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