Supplementary Information

Structure-function analysis for development of peptide inhibitors for a Gram positive quorum sensing system

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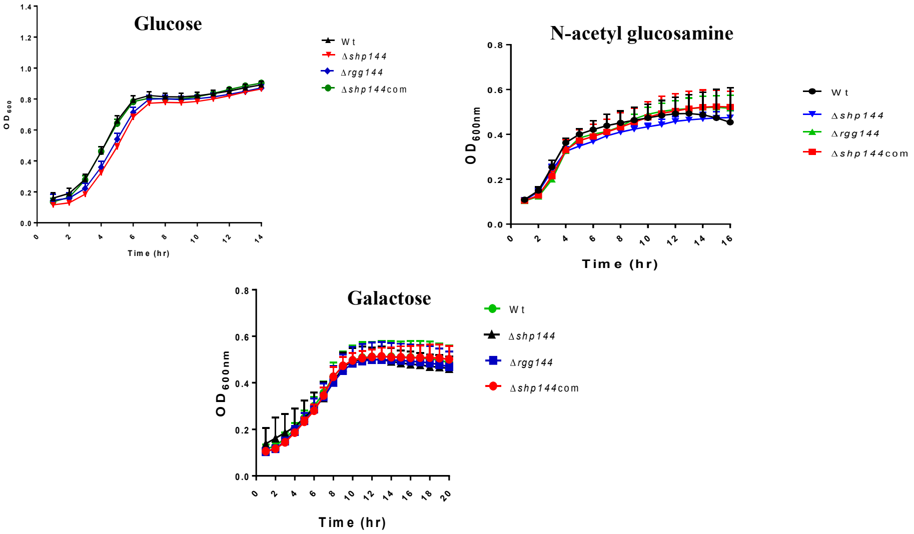
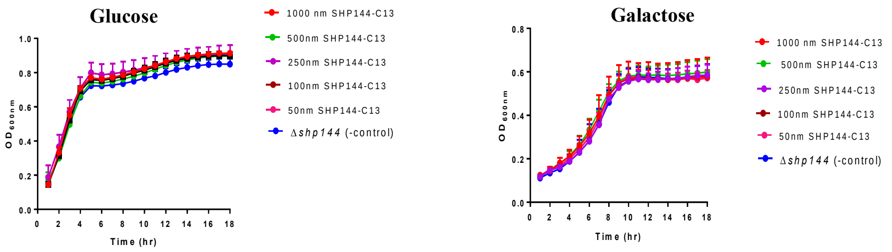
Key words: Rgg transcriptional regulators, *Streptococcus pneumoniae*, quorum sensing, structure-function, inhibitor design

Running title: Inhibition of a Gram positive quorum sensing system

(A)


Glucose
Wt
Δshp144
Δrgg144
Δshp144com
OD 600
Time (hr)
N-acetyl glucosamine
Wt
Δshp144
Δrgg144
Δshp144com
OD 600nm
Time (hr)
Galactose
Wt
Δshp144
Δrgg144
Δshp144com
OD 600nm
Time (hr)

(B)


Glucose
1000 nm SHP144-C13
500nm SHP144-C13
250nm SHP144-C13
100nm SHP144-C13
50nm SHP144-C13
Δshp144 (-control)
OD 600nm
Time (hr)
Galactose
1000 nm SHP144-C13
500nm SHP144-C13
250nm SHP144-C13
100nm SHP144-C13
50nm SHP144-C13
Δshp144 (-control)
OD 600nm
Time (hr)

(C

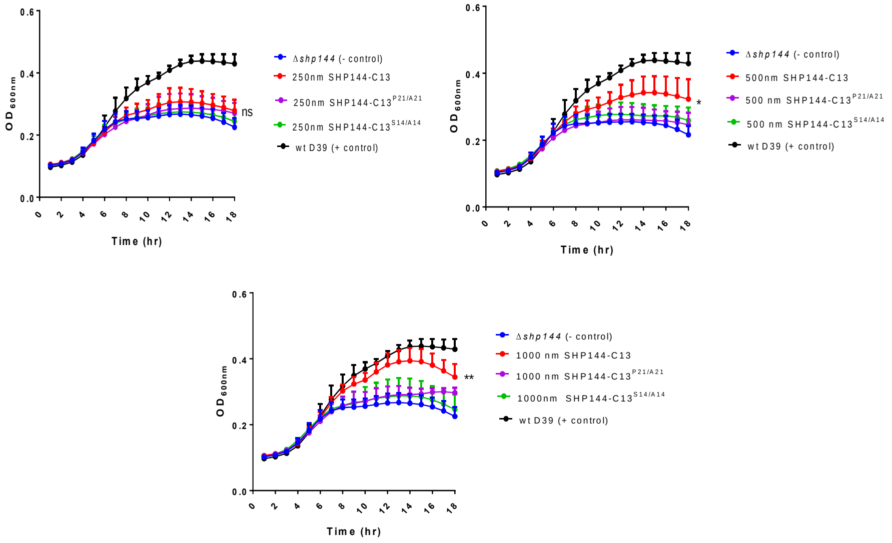

Δshp144 (- control)
250nm SHP144-C13
250nm SHP144-C13 P21/A21
250nm SHP144-C13 S14/A14
wt D39 (+ control)
ns
OD 600nm
Time (hr)
Δshp144 (- control)
500nm SHP144-C13
500 nm SHP144-C13 P21/A21
500 nm SHP144-C13 S14/A14
wt D39 (+ control)
*
OD 600nm
Time (hr)
Δshp144 (- control)
1000 nm SHP144-C13
1000 nm SHP144-C13 P21/A21
1000nm SHP144-C13 S14/A14
wt D39 (+ control)
**
OD 600nm
Time (hr)

Figure S1: (A) Pneumococcal growth curves in CDM supplemented with 55nM of desired sugar (glucose, N-acetylglucosamine and galactose). Error bars indicate standard error of the mean of at least three independent experiments each with three replicates. No significant difference has been seen by using those selected sugars. **(B)** Growth curve of Δ*shp144 showing* inability of unmodified SHP144-C13 to restore mutant growth properties in media containing either glucose or galactose. Comparisons are made relative to Δshp144 culture without peptide. **(C)** Investigation the specificity of functional complementation of Δshp144 growth by using modified SHP144. Various concentrations of native and modified SHP144-C13 (P 21A and S14A) were used to reconstitute pneumococcal growth defect on mannose. No growth complementation could be observed by using modified peptides. This experiment was done for three independent biological samples and each with three replicates. *p<0.05, ** p<0.01 compared to Δshp144 in the absence of synthetic peptide.

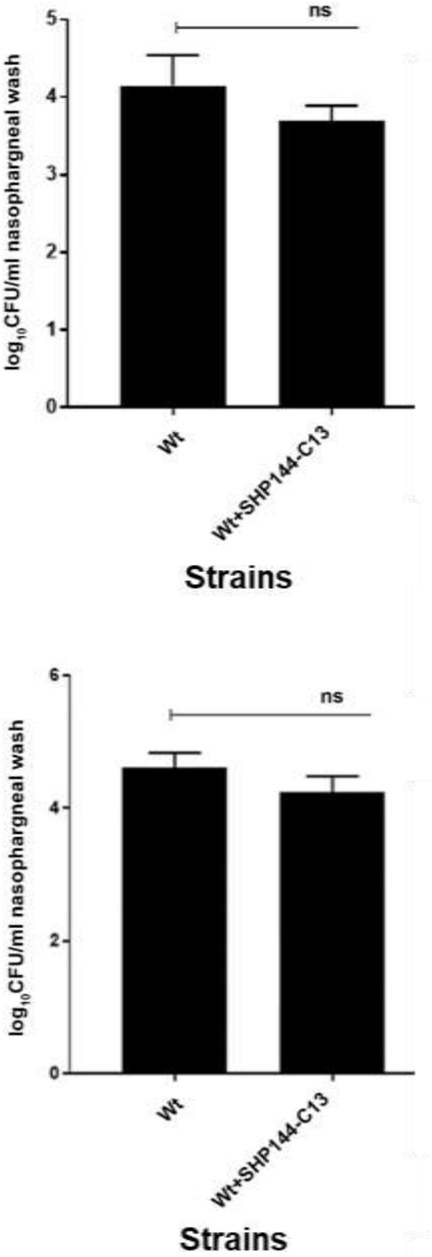


Figure S2: Identification the impact of SHP144 synthetic peptides on pneumococcal colonisation in murine model. (A-B) mice infected intranasally either with 2.5x10^5 CFU/mouse of pneumococcal D39 wild type (control) or the pneumococcal inoculum containing 200 µM of SHP144-C13 peptide. The cohort reciving peptide had additional doses of peptide at predetermined times (24, 48 and 72h post infection) whereas control group received only 20 µl PBS. The mice at day 0 and 5 were sacrificed by cervical dislocation and nasal washes were obtained and processed as described before. Error bars show the standard error of the mean. A two-tailed unpaired student's t-test and one-way ANOVA followed by Tukey's multiple comparison tests were applied to compare the bacterial counts with control group (n=5 for each group, 'ns' not significant).

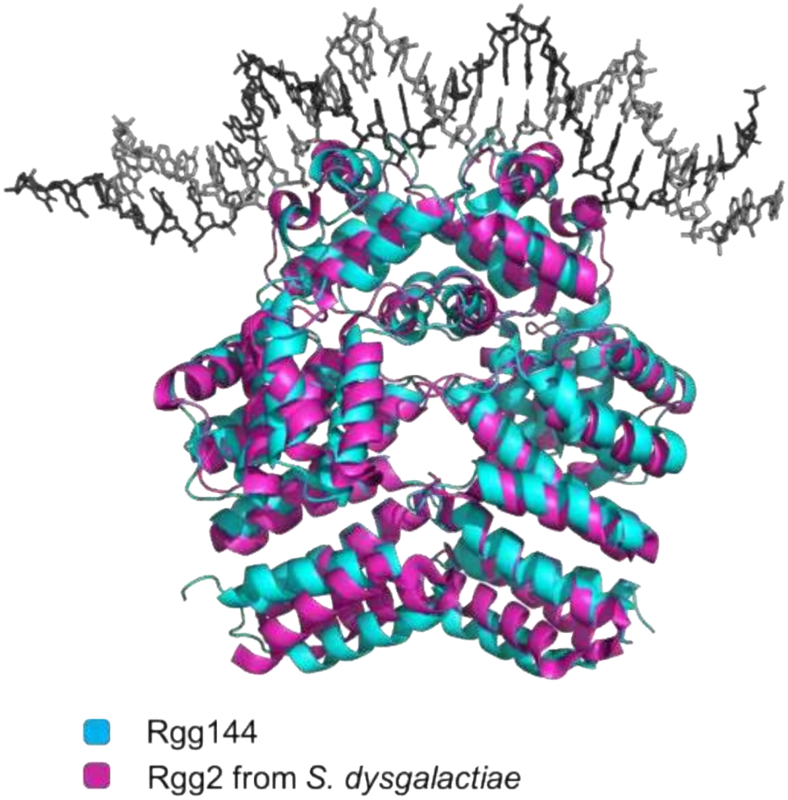

🟦 Rgg144
🟪 Rgg2 from *S. dysgalactiae*

Figure S3: Overlay of Rgg144 with the DNA bound form of Rgg2 from *S. dysgalactiae* (PDB: 6W1A). The structure of the unbound form of Rgg144 is broadly comparable to the structure of the DNA bound form of Rgg2 suggesting a similar mode of action in which binding of the SHP displaces the helix-turn-helix domains of the Rgg(8).

Table S1: Data collection and refinement statistics.

	Rgg144
PDB ID	7ZCV
Wavelength, (Å)	0.9795
Resolution range	32.51 – 1.90 (1.94 - 1.90)*
Space group	P 21 21 21
Unit cell, *a, b, c* (Å)	75.5, 80.9,106.5
α, β, γ (°)	90, 90, 90
Total reflections	313591 (20704)
Unique reflections	52047 (5148)
Multiplicity	6.0 (6.2)
Completeness (%)	99.9 (99.9)
Mean I/σI	12.3 (2.0)
Wilson B-factor	34.3
R-sim	0.080 (1.031)
R-pim	0.044 (0.412)
CC1/2	0.998 (0.526)
Refinement	
Reflections	52046 (5148)
R-work	0.1865
R-free	0.2183
Number of atoms	5121
macromolecules	4749
solvent	372
RMS deviations	
Bond lengths (Å)	0.011
Bond angles (°)	1.08
Ramachandran favored (%)	99.3
Average B-factor	44.3
macromolecules	44.2
solvent	47.0

*Statistics for the highest-resolution shell are shown in parentheses.

Table S2: Bacterial strains and plasmids used in this study.

Strain/Plasmid	Description	Source
***S. pneumoniae* D39**	Serotype 2	The National Collection of Type Cultures, London, UK Strain NTCC 7466
Δshp144	D39Δshp144; Spec^R	Reference [1]
Δshp144com	D39; shp144 + Δshp144: Spec^R; Kan^R	This study
Δshp144com$^{S14/A14}$	D39; shp144$^{S14/A14}$ + Δshp144: Spec^R; Kan^R	This study
Δshp144Com$^{E15/A15}$	D39; shp144$^{E15/E15}$ + Δshp144: Spec^R; Kan^R	This study
Δshp144Com$^{W16/A16}$	D39; shp144$^{W16/A16}$ + Δshp144: Spec^R; Kan^R	This study
Δshp144Com$^{V17/A17}$	D39; shp144$^{V17/A17}$ + Δshp144: Spec^R; Kan^R	This study
Δshp144Com$^{I18/A18}$	D39; shp144$^{I18/A18}$ + Δshp144: Spec^R; Kan^R	This study
Δshp144Com$^{V19/A19}$	D39; shp144$^{V19/A19}$ + Δshp144: Spec^R; Kan^R	This study
Δshp144Com$^{I20/A20}$	D39; shp144$^{I20/A20}$ + Δshp144: Spec^R; Kan^R	This study
Δshp144Com$^{P21/A21}$	D39; shp144$^{P21/A21}$ + Δshp144: Spec^R; Kan^R	This study
Δshp144Com$^{F22/A22}$	D39; shp144$^{F22/A22}$ + Δshp144: Spec^R; Kan^R	This study

Table S3: Oligonucleotides primers used in this study*.

Primers	**Sequence (5’–3’)**
shp144com/F	CATG**CCATGG**CAAGTACAGTATAACACGAAA
shp144com/R	ACG**GGATCC**CCTTTTTGAATTGCGTTTTCAGCA
Shp144S14/F	CCTACTTATTGCGGAGTGGGTTA
Shp144S14/R	TAACCCACTCCGCAATAAGTAGG
Shp144 E15/F	CTTATTTCGGCGTGGGTTATTGTCA
Shp144 E15/R	TGACAATAACCCACGCCGAAATAAG
Shp144 W16/F	TATTTCGGAGGCGGTTATTGTCATTC
Shp144 W16/R	GAATGACAATAACCGCCTCCGAAATA
Shp144 V17/F	TCGGAGTGGGCTATTGTCATTCCAT
Shp144 V17/R	ATGGAATGACAATAGCCCACTCCGA
Shp144 I18/F	GGAGTGGGTTGCTGTCATTCCATTTT
Shp144 I18/R	AAAATGGAATGACAGCAACCCACTCC
Shp144 V19/F	TGGGTTATTGCCATTCCATTTTTAA
Shp144 V19/R	TTAAAAATGGAATGGCAATAACCCA
Shp144 I20/F	GGTTATTGTCGCTCCATTTTTAAC
Shp144 I20/R	GTTAAAAATGGAGCGACAATAACC
Shp144 P21/F	TATTGTCATTGCATTTTTAACTAAT
Shp144 P21/R	ATTAGTTAAAAATGCAATGACAATA
Shp144 F22/F	TGTCATTCCAGCTTTAACTAATCT
Shp144 F22/R	AGATTAGTTAAAGCTGGAATGACA
Shp144 L 23/F	CATTCCATTTGCAACTAATCTATAAG
Shp144 L 23/R	CTTATAGATTAGTTGCAAATGGAATG
Shp144 T 24/F	TCCATTTTTAGCTAATCTATAAGTT
Shp144 T 24/R	AACTTATAGATTAGCTAAAAATGGA
Shp144 N 25/ F	TTTTTAACTGCTCTATAAGTTCTT
Shp144 N 25/R	AAGAACTTATAGAGCAGTTAAAAA
Shp144 L 26/F	TAACTAATGCATAAGTTCTTTATATTG
Shp144 L 26/R	CAATATAAAGAACTTATGCATTAGTTA
Nco1- shp144/F	AACACACGAGGTGCTA**CCATGG**CAACTCAGCTTCTGTCAATTCC
BamH1-shp144/R	CCATTAAAAATCAAAC**GGATCC**CTTATCAGAACTCATGGAGCGA
Mal/F	GCTTGAAAAGGAGTATACTT
pCEP/R	AGGAGACATTCCTTCCGTATC
Fusion seq/UF	CTACTTGGAGCCACTATCGA
Fusion seq/DR	AGGCGATTAAGTTGGGTAAC
Spd0144-Full/F	TACTTCCAATCCATGATTGAAAAAATGGAACTGGG
Spd0144-Full/R	TATCCACCTTTACTGTCAATCTATAAGTTCTTTATATT
T7 Promoter-F (PNACL)	TAATACGACTCACTATAGGG
pLEICS-01-Seq-R (PNACL)	ATTAACATTAGTGGTGGTGGT

*Bold typeface indicates incorporated restriction sites whereas underlined letters represents homological regions of pCEP and pLEICS-01, respectively.

Table S4: List of SHP144-C13 synthetic peptides used in this study. The letter (A) represents the replacement of individual amino acid of SHP144-C13 with alanine whereas (YSATH) indicates the changing of five amino acids (W V I V I) of SHP144-C13.

Name	**Sequence**
FITC-SHP144-C13	FITC- S E W V I V I P F L T N L- COOH
FITC-SHP144-C13$^{S14/A14}$	FITC- **A** E W V I V I P F L T N L- COOH
FITC-SHP144-C13$^{E15/A15}$	FITC- S **A** W V I V I P F L T N L- COOH
FITC-SHP144-C13$^{W16/A16}$	FITC- S E **A** V I V I P F L T N L- COOH
FITC-SHP144-C13$^{V17/A17}$	FITC- S E W **A** I V I P F L T N L- COOH
FITC-SHP144-C13$^{I18/A18}$	FITC- S E W V **A** V I P F L T N L- COOH
FITC-SHP144-C13$^{V19/A19}$	FITC- S E W V I **A** I P F L T N L- COOH
FITC-SHP144-C13$^{I20/A20}$	FITC- S E W V I V **A** P F L T N L- COOH
FITC-SHP144-C13$^{P21/A21}$	FITC- S E W V I V I **A** F L T N L- COOH
FITC-SHP144-C13$^{F22/A22}$	FITC- S E W V I V I P **A** L T N L- COOH
FITC-SHP144-C13$^{L23/A23}$	FITC- S E W V I V I P F **A** T N L- COOH
FITC-SHP144-C13$^{T24/A24}$	FITC- S E W V I V I P F L **A** N L- COOH
FITC-SHP144-C13$^{N25/A25}$	FITC- S E W V I V I P F L T **A** L- COOH
FITC-SHP144-C13$^{L26/A26}$	FITC- S E W V I V I P F L T N **A**- COOH
FITC-NEC-C13	FITC-SE**YSATH**PFLTNL - COOH
SHP144-C13	NH2- S E W V I V I P F L T N L- COOH
SHP144-C13$^{S14/A14}$	NH2- **A** E W V I V I P F L T N L- COOH
SHP144-C13$^{P21/A21}$	NH2- S E W V I V I **A** F L T N L- COOH