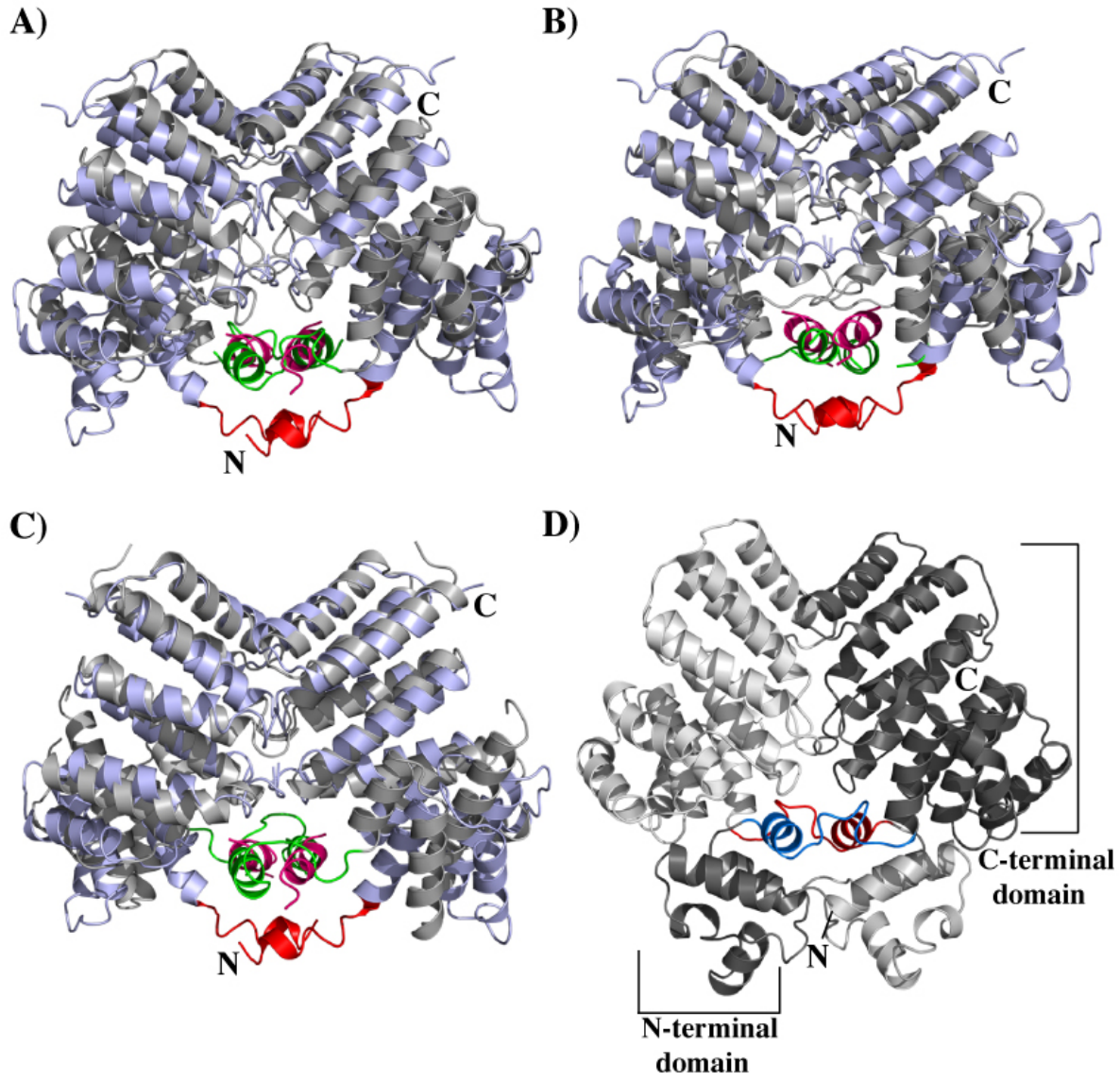
Supplementary figure S1

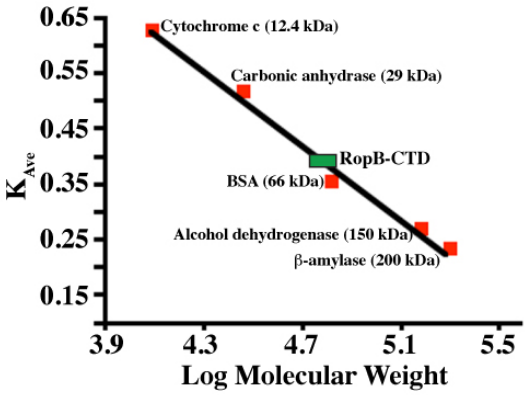


Supplementary figure S1. Comparison of the relative position of the bridging helix α1 (α4 in full-length structures) between RopB-CTD and the full-length structures of RRNPP family regulators. Superposition of RopB-CTD dimer (blue) with the analogous structural elements from PrgX **(A)** from *E. faecalis* (PDB code: 2AXZ), Rgg-like protein **(B)** from *L. monocytogenes* (PDB code: 4RYK), and Rgg2 **(C)** from *S. dysgalactiae* (PDB code: 4YV9). The dimeric structures of RopB structural homologs are colored in grey. The helix α1 of RopB-CTD is colored in red, whereas the bridging helices of the structural homologs are colored in green. The helix α1 from the symmetry mates in RopB crystal are shown and colored in pink. The N- and C-termini of RopB-CTD are marked as N and C, respectively. Structural superposition was performed with "LSQKAB". D) The interdomain interactions between the bridging helix of one subunit and the C-terminal domain of the second subunit of a dimer, as observed in the full-length structure of PrgX from *E. faecalis* (PDB code: 2AXZ). Individual subunits of a PrgX

dimer is colored in light and dark grey and the N-terminal DNA-binding and C-terminal oligomerization/regulatory domain are labeled. The bridging helices of each subunit are color-coded (in blue and pink).

Supplementary figure S2

A)

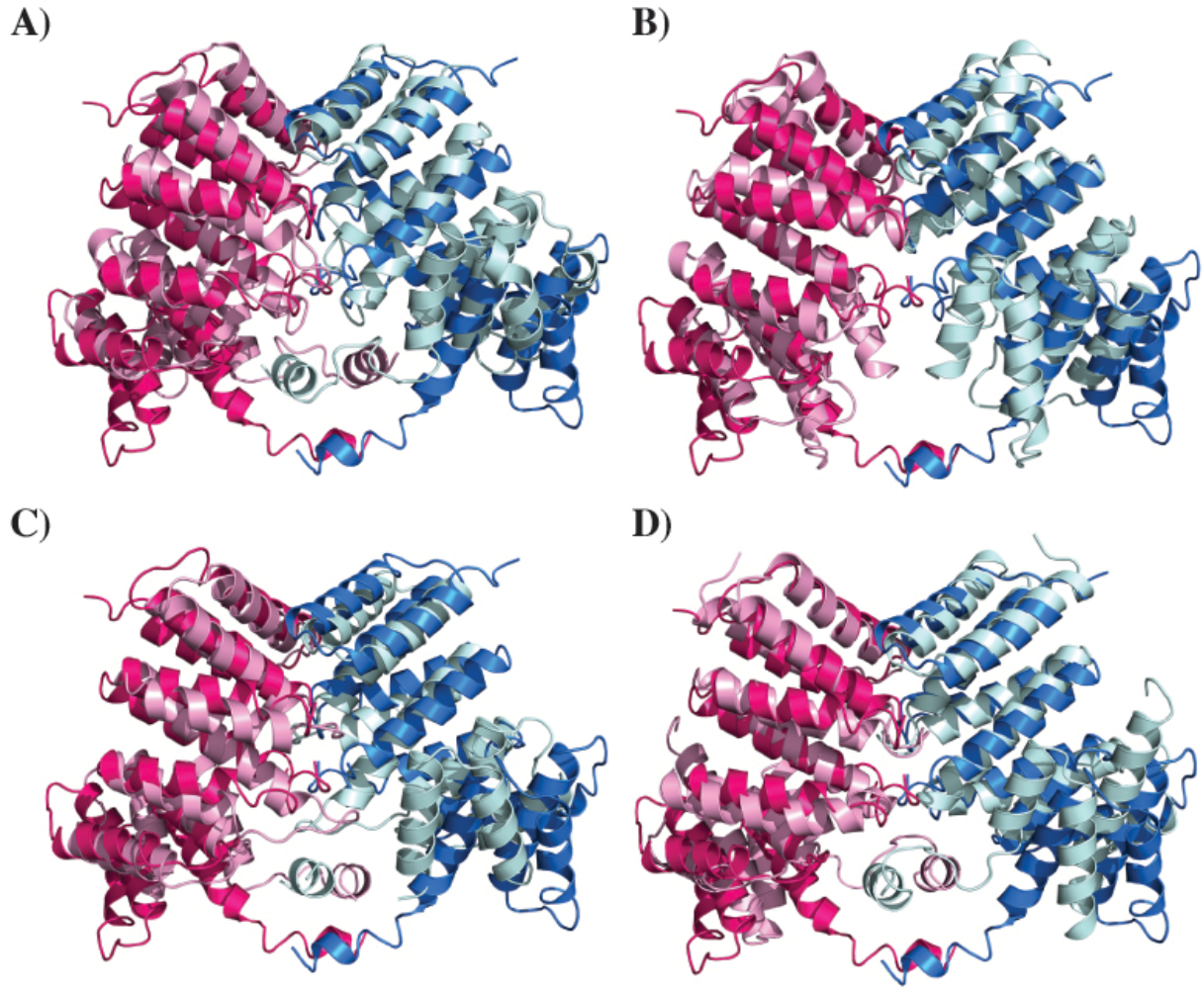


B)

	Oligomerization state	Predicted MW (in kDa)	Experimentally derived MW (in kDa)
RopB-CTD	Monomer	27.9	60.3
	Dimer	55.8	

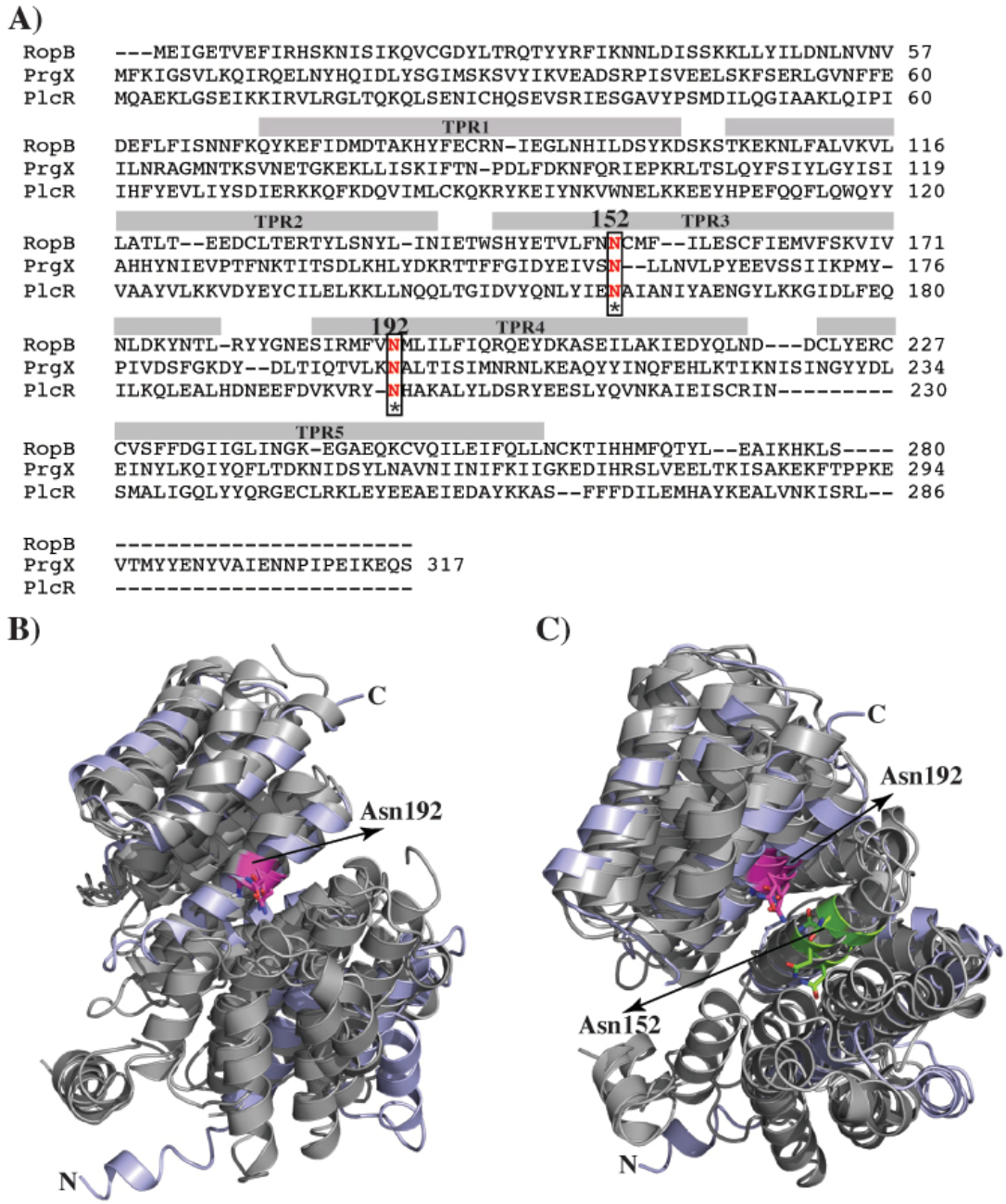
Supplementary figure S2. Assessment of the oligomerization state of RopB-CTD in solution by size exclusion chromatography. A) The linear fit of the elution volumes (K_{Ave}) of five protein molecular weight standards, cytochrome c (12.4 kDa), carbonic anhydrase (29 kDa), bovine serum albumin (BSA, 66 kDa), alcohol dehydrogenase (150 kDa), and β-amylase (200 kDa), to their log molecular weight is shown as a black line on the graph. The elution volume (K_{Ave}) of the purified RopB-CTD was plotted on the graph (green rectangle). B) RopB-CTD exists as a dimer in solution. The theoretical molecular weights of different oligomeric states of RopB-CTD were calculated based on their amino acid sequence, and the experimentally calculated molecular weight of RopB-CTD based on the elution profile in a superdex-200 size exclusion chromatography are shown.

Supplementary figure S3



Supplementary figure S3. Structural homology shared by RopB-CTD with the structurally characterized members of RRNPP family regulators. Superposition of RopB-CTD dimer with the analogous structural elements from PrgX **(A)** from *E. faecalis* (PDB code: 2AXZ), PlcR **(B)** from *B. thuringiensis* (PDB code: 2QFC), Rgg2 **(C)** from *S. dysgalactiae* (PDB code: 4YV9) and Rgg-like protein (D) from *L. monocytogenes* (PDB code: 4RYK). Structural superposition was performed with "LSQKAB" program. Individual subunits of RopB-CTD dimer are colored in dark blue and pink, whereas the subunits of structural homologs are shaded in light blue and pink.

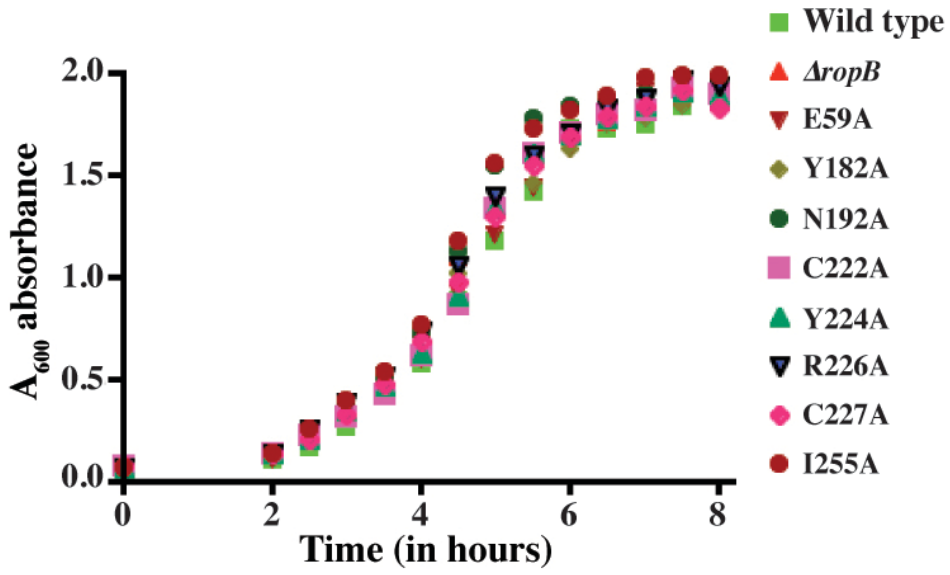
Supplementary figure S4



Supplementary figure S4. Conserved asparagines in the ligand-binding pocket of structurally characterized members of RRNPP family regulators. A) Amino acid sequence alignment of RopB, PlcR from *B. thuringiensis*, and PrgX from *E. faecalis*. The positions of TPR motifs derived from the RopB-CTD structure are indicated above the alignment. The conserved asparagines that interact with the cognate peptides in the co-crystal structures of peptide-bound PrgX and PlcR are boxed and marked in red. The alignment was carried out with ClustalW. Structural superposition of the C-terminal domain of RopB with the analogous residues from the

structures of PrgX (PDB code: 2AXZ), PlcR (PDB code: 2QFC), Rgg2 (PDB code: 4YV9), and Rgg-like protein from *L. monocytogenes* (PDB code: 4RYK). The location of the side chains of asparagines, Asn 192 **(B and C)** and Asn 152 **(C)** is highlighted in pink and green, respectively, and labeled. The side chains are displayed as ball and stick representation. The N- and C-termini of RopB-CTD are labeled as N and C, respectively. Structural superposition was performed with LSQKAB.

Supplementary figure S5



Supplementary figure S5. Growth curve of indicated strains in THY broth.

Supplementary table S1.
Selected crystallographic data and statistics.

Data Collection and Phasing			
Dataset	**SeMet**		**Native**
Wavelength (l)	0.97971	0.957	1.12
Resolution (Å)	50.0 – 3.8		81.0 – 3.5
R_{sym} [a]	0.097 (0.5)[b]	0.094 (0.5)	0.070 (0.98)
I/s(I)	10.7 (3.7)	11.6 (3.9)	14.7 (2.2)
Total Reflections (#)	76335	75984	61325
Unique Reflections (#)	9357	9337	11346
Completeness (%)	100 (99.9)	100 (99.9)	97.8 (95.3)
Selenium sites (identified/total no of sites)	10/12		
Overall Figure of Merit[c]	0.35		
Refinement Statistics			
Resolution Range (Å)			81.0-3.5
R_{work}/R_{free}(%)[d]			27.6/31.3
Atoms (#)			
Protein			3704
B factors (Å^2)			130.5
rmsd			
Bond lengths (Å)			0.004
Bond angles (°)			0.865
Ramachandran analysis			
Most favoured (%)			96.33
Add. allowed (%)			3.67
Gen. allowed (%)			0.0
Disallowed (%)			0.0

[a]$R_{sym} = \sum\sum|I_{hkl}-I_{hkl(j)}|/ \sum I_{hkl}$, where $I_{hkl(j)}$ is the observed intensity and I_{hkl} is the final average intensity value. [b]Values in parentheses are for the highest resolution shell. [c]Figure of Merit = $<|SP(a)e^{ia}/SP(a)|>$, where a is the phase and P(a) is the phase probability distribution. [d]$R_{work} = S||F_{obs}| - |F_{calc}||/S|F_{obs}|$ and $R_{free} = S||F_{obs}| - |F_{calc}||/S|F_{obs}|$; where all reflections belong to a test set of 5% randomly selected reflections.

Supplementary table S2. Bacterial strains and plasmids used in this study.

Strain or plasmid	Description	Reference
Strains		
MGAS10870	Invasive isolate, serotype M3	(1)
MGAS10870Δ*ropB*	MGAS10870Δ*ropB*::*aad9*	(2)
MGAS10870Δ*speB*	MGAS10870Δ*speB*::*aad9*	(4)
Plasmids		
pET21b	Overexpression vector for C-terminally hexa-histidine tagged recombinant proteins	
pJL	Low-copy number plasmid capable of replication in GAS and *Escherichia coli*, Cm^+	(3)

Supplementary table S3. Primers and probes used in this study

Primer	Sequence 5'-3'	Application
E59A Top	TGTGAACGTTGAC**GCT**TTTCTGTTCATCAG	Site directed mutagenesis to introduce E59A
E59A Bottom	CTGATGAACAGAAAAGCGTCAACGTTCACA	Site directed mutagenesis to introduce E59A
Y182A Top	AATACCCTAAGGTAT**GCT**GGGAATGAATCGATTC	Site directed mutagenesis to introduce Y182A
Y182A Bottom	GAATCGATTCATTCCCAGCATACCTTAGGGTATT	Site directed mutagenesis to introduce Y182A
N192A Top	ATTCGGATGTTTGTC**GCT**ATGTTGATTTTG	Site directed mutagenesis to introduce N192A
N192A Bottom	CAAAATCAACATAGCGACAAACATCCGAAT	Site directed mutagenesis to introduce N192A
C222A Top	ATCAGCTAAATGATGAT**GCT**TTATATGAACGGTG	Site directed mutagenesis to introduce C222A
C222A Bottom	CACCGTTCATATAAAGCATCATCATTTAGCTGAT	Site directed mutagenesis to introduce C222A
Y224A Top	ATGATGATTGCTTA**GCT**GAACGGTGTTGTG	Site directed mutagenesis to introduce Y224A
Y224A Bottom	CACAACACCGTTCAGCTAAGCAATCATCAT	Site directed mutagenesis to introduce Y224A
R226A Top	ATTGCTTATATGAA**GCT**TGTTGTGTGTCT	Site directed mutagenesis to introduce R226A
R226A Bottom	AGACACACAACAAGCTTCATATAAGCAAT	Site directed mutagenesis to introduce R226A
C227A Top	TGCTTATATGAACGG**GCT**TGTGTGTCTTTTTTTG	Site directed mutagenesis to introduce C227A
C227A Bottom	CAAAAAAAGACACACAAGCCCGTTCATATAAGCA	Site directed mutagenesis to introduce C227A
I255A Top	TTCAAATTCTGGAA**GCC**TTTCAGCTGCTG	Site directed mutagenesis to

		introduce I255A
I255A Bottom	CAGCAGCTGAAAGGCTTCCAGAATTTGAA	Site directed mutagenesis to introduce I255A
tufA qRTFwd	CAACTCGTCACTATGCGCACAT	qRT-PCR analysis of *tufA*
tufA qRTRev	GAGCGGCACCAGTGATCAT	qRT-PCR analysis of *tufA*
tufA probe	CTCCAGGACACGCGGACTACGTTAAAAA	qRT-PCR analysis of *tufA*
speB qRTFwd	CAACTCGTCACTATGCGCACAT	qRT-PCR analysis of *speB*
speB qRTRev	GAGCGGCACCAGTGATCAT	qRT-PCR analysis of *speB*
speB probe	CTCCAGGACACGCGGACTACGTTAAAAA	qRT-PCR analysis of *speB*
ropB qRTFwd	CAACTCGTCACTATGCGCACAT	qRT-PCR analysis of *ropB*
ropB qRTRev	GAGCGGCACCAGTGATCAT	qRT-PCR analysis of *ropB*
ropB probe	CTCCAGGACACGCGGACTACGTTAAAAA	qRT-PCR analysis of *ropB*

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3. **Li, J., D. L. Kasper, F. M. Ausubel, B. Rosner, and J. L. Michel.** 1997. Inactivation of the α C protein antigen gene, *bca*, by a novel shuttle/suicide vector results in attenuation of virulence and immunity in group B *Streptococcus*. Proc. Natl. Acad. Sci. USA **94:**13251-13256.
4. **Shelburne III, S. A., R. J. Olsen, N. Makthal, N. G. Brown, P. Sahasrabhojane, E. M. Watkins, T. Palzkill, J. M. Musser, and M. Kumaraswami.** 2011. An amino-terminal signal peptide of Vfr protein negatively influences RopB-dependent SpeB expression and attenuates virulence in *Streptococcus pyogenes*. Mol. Microbiol. **82:**1481-1495.