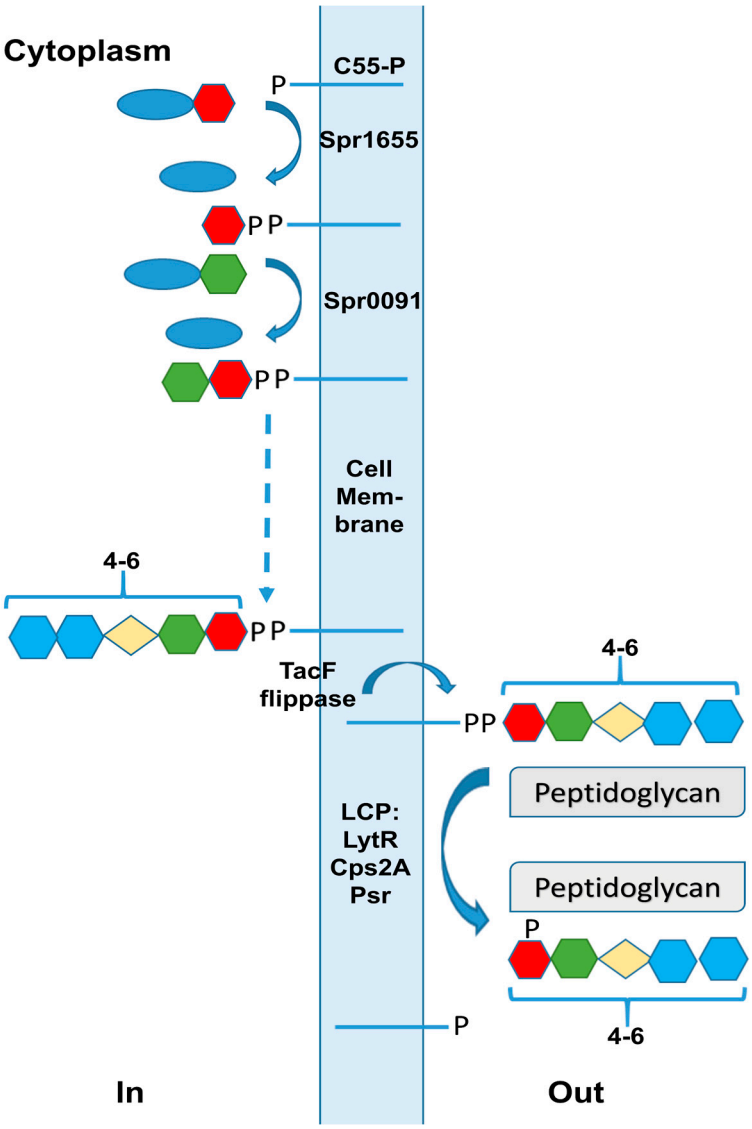

Figure S1. Teichoic acid biosynthesis pathway suggested for *S. pneumoniae* in [1]. Blue ellipse: UMP; red hexagon: AATGal; green hexagon: glucose; yellow tetragon: ribitol; blue hexagons: N-acetyl- galactose-amine with phosphoryl choline. For simplicity, only the first two and last two reactions in the pathway are shown. Spr1655 and Spr0091—-enzymes proposed to catalyze the initial steps, while TacF and LCP are implicated in the final stages of the pathway.

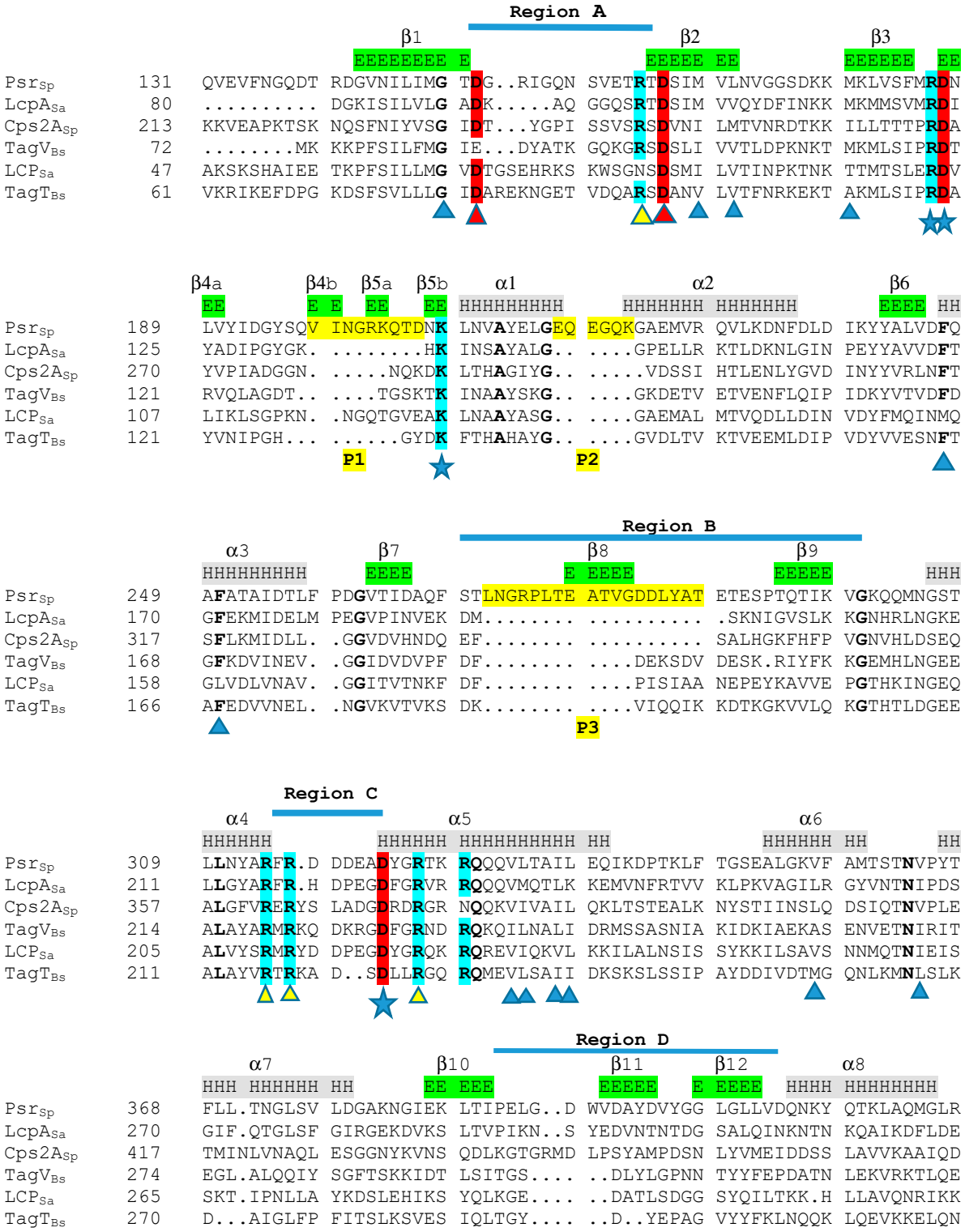

Figure S2. Multiple sequence alignment of the LCP domain of Psr$_{Sp}$ with the five best structural hits found by DALI demonstrates low sequence conservation.

A DALI search based on the crystal structure of PsrSp gave LcpA$_{Sa}$ from *Staphylococcus aureus* (6uex.pdb), the LCP domain of Cps2A from *S. pneumoniae* (4de8.pdb), TagV from *B. subtilis* (6uf5.pdb), TagT from *B. subtilis* (6uf5.pdb), and the LCP protein from *S. agalactiae* (3okz.pdb) as the five best hits. ~~Sequence~~ The sequence alignment of these six proteins reveals low sequence

conservation. The secondary structure of Psr_{Sp} and the localization of the A, B, C, D regions are presented above the sequences. The P1–P3 insertions in Psr_{Sp} are marked in yellow. The ~~15~~ fifteen residues that are strictly conserved among all six structures are in bold. The essential acidic residues within the active site are in red, while basic residues are in blue. The aspartic acid residues involved in Mg^{2+} binding are marked with red triangles, while the arginine residues that are essential for pyrophosphate binding are marked with yellow triangles. Residues lining the tunnel for the polyprenyl moiety of the substrate are marked with blue triangles. Residues that can be essential for peptidoglycan binding are marked with stars.

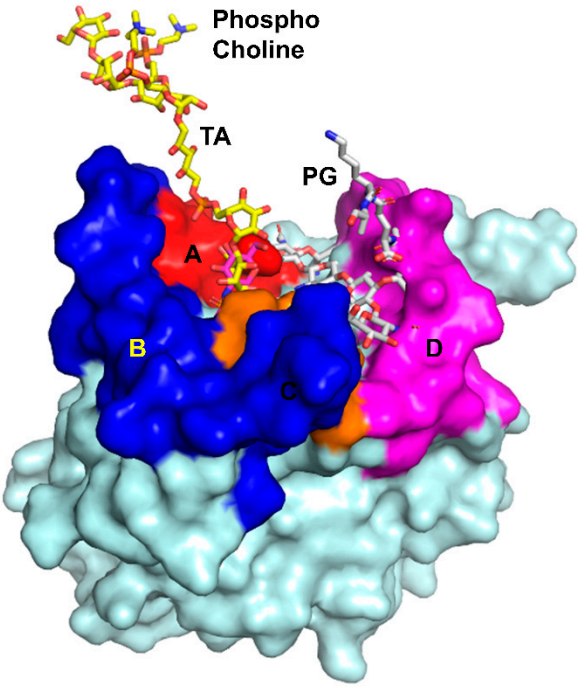

Figure S3. Hypothetical molecular model of the undecaprenyl-WTA/PG/Psr$_{Sp}$ complex demonstrates that regions A and B can interact with a large section of the first RU of the polysaccharide, whereas region D is close to the peptidoglycan.

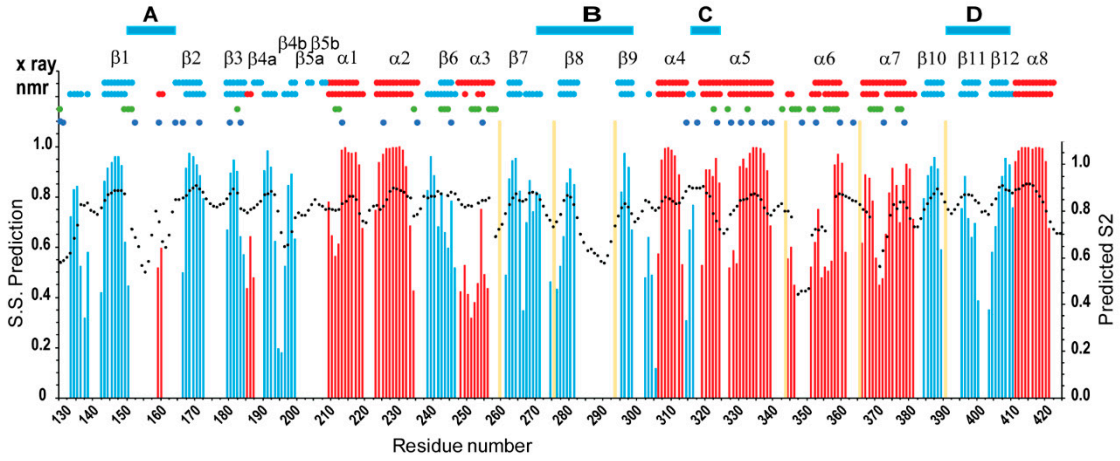

Figure S4. The secondary structures of PsrSp obtained from NMR concord with those derived from the crystal structure of PsrSp. The secondary structure prediction (S.S. Prediction) index, predicted order parameter S2 were extracted from the CS of the ^{1}H(N), ^{15}N, ^{13}Cα, ^{13}Cβ and ^{13}C′ backbone resonances by TALOS-N. The S.S. ~~Prediction~~ prediction indexes versus the amino acid sequences are presented on the left side of the panel. Red and blue bars indicate α-helices/turns and β-strands, respectively. Predicted S2 are presented on the right side of the panels~~,~~ and are indicated by black dots. Schematic representations of the X-ray- and NMR-determined secondary structures are shown on the top of the panels. Blue and green circles represent the amino acids where either only NH or all resonances were not assigned, respectively. The range of the loop regions A, B, C, and D are shown on the top of the panels.

Table S1. **Data collection and refinement statistics for Psr_{Sp}**

Data collection	
Space group	P 3 2 1
Cell dimensions	
a, *b*, *c* (Å)	130.63 130.63 100.58
α, β, γ (°)	90.0 90.0 120.0
Resolution (Å)	45.9-2.15 (2.30-2.15)
R_{merge}	0.114 (1.099)
I / σI	13.7 (1.7)
Completeness spherical (%)	64.2 (17.3)
Completeness ellipsoidal (%)	94.5 (63.2)
Redundancy	9.9 (6.2)
Refinement	
Resolution (Å)	45.9-21.5 (2.20-2.15)
No. reflections	33 065 (284)
R_{work} / R_{free}	0.20/0.26 (0.32/0.31)
No. atoms	7082
Protein	6856
Water	226
B-factors (Å^2)	
Protein	52.9
Water	40.7
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.481
Diffraction source	MAX IV/BioMAX
PDB ID	9GN3

Values in parentheses are for highest-resolution shell.

1. Denapaite, D.; Brückner, R.; Hakenbeck, R.; Vollmer, W. Biosynthesis of teichoic acids in Streptococcus pneumoniae and closely related species: lessons from genomes. *Microb Drug Resist* **2012**, *18*, 344-358, doi:10.1089/mdr.2012.0026.