SUPPLEMENTAL INFORMATION

A catalytically essential motif in the external loop 5 of the bacterial oligosaccharyltransferase PglB*

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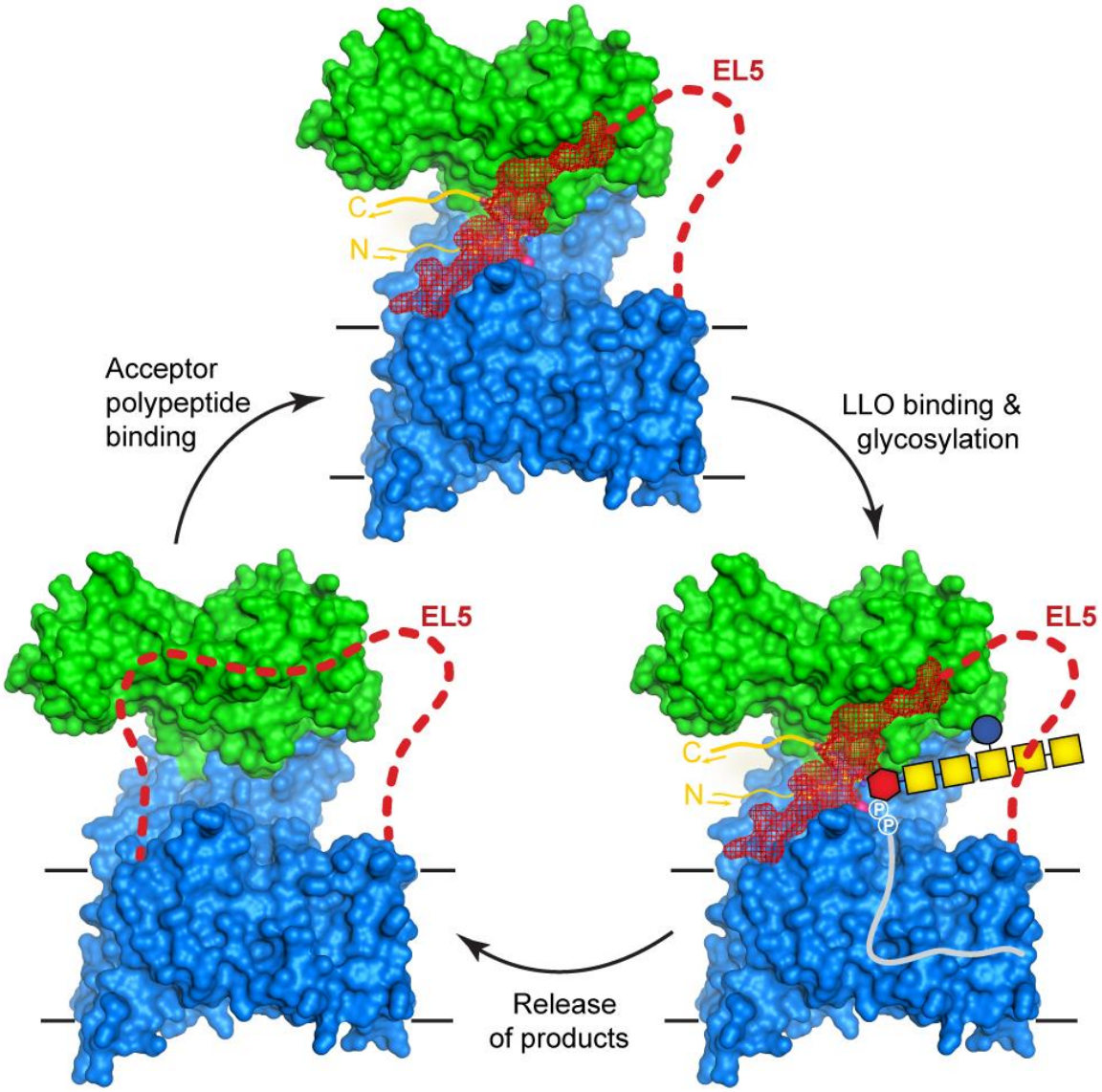
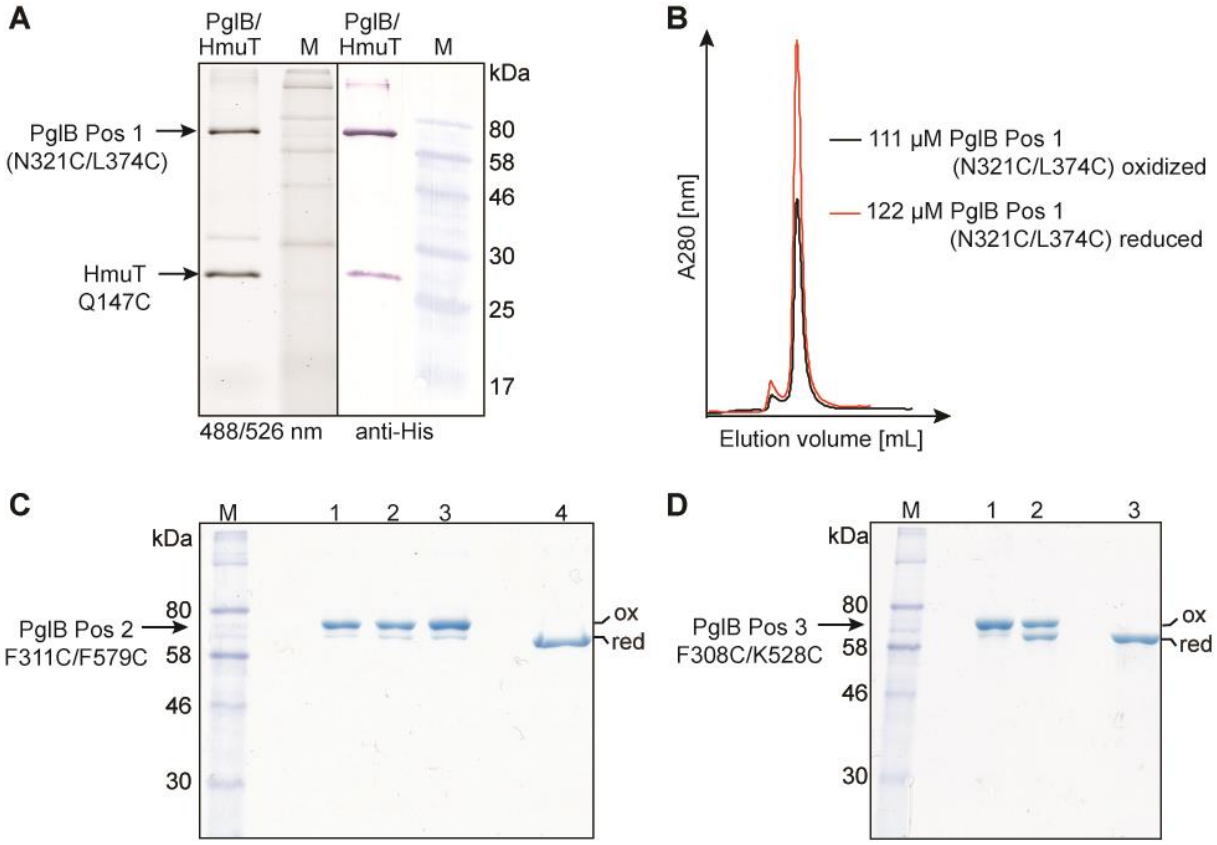
SUPPLEMENTAL FIGURES

Supplemental Figure S1



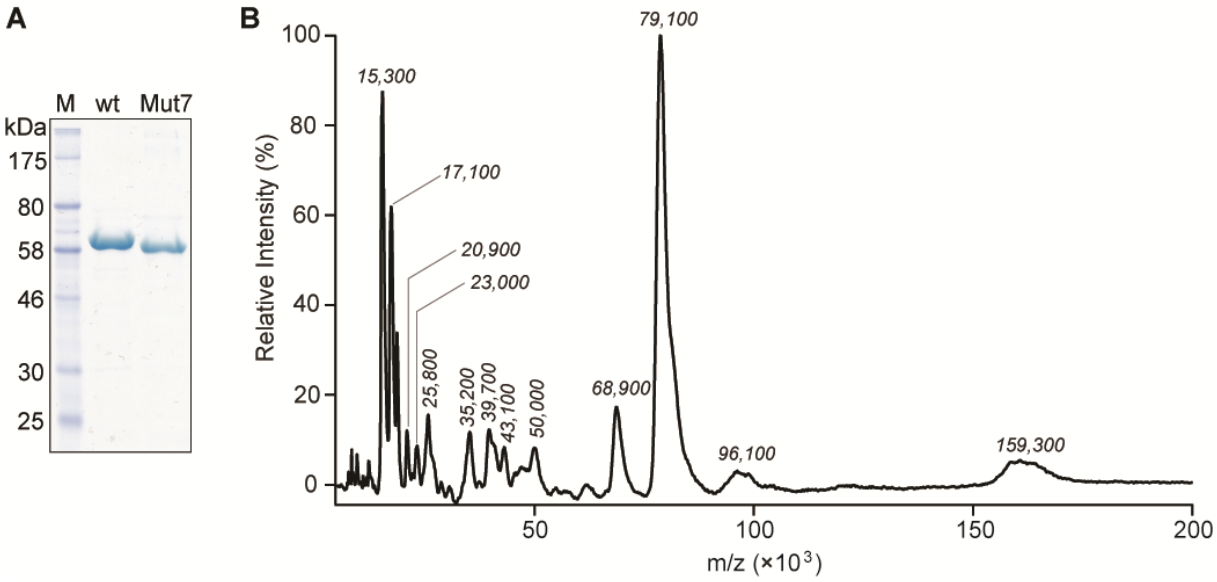
Supplemental Figure S1: Proposed glycosylation cycle of PglB. Surface representation of PglB from *C. lari* (PDB code 3RCE) with the transmembrane domain colored in blue, periplasmic domain in green and external loop 5 (EL5) in red. The bound acceptor peptide is in ball and stick and yellow lines indicate the N and C termini. The disordered part of EL5 is indicated by a red dashed line. The top state represents the existing crystal structure of PglB with acceptor peptide bound and the C-terminal half of EL5 ordered and engaged. The bottom-left state reflects PglB in the ground state with no substrates bound and EL5 completely disordered. The bottom-right state represents a reaction intermediate with acceptor peptide substrate and LLO donor substrate bound to PglB. Red hexagon: diNAcBac; yellow square: N-acetyl-galactosamine; blue circle: glucose; circled P: phosphate group; grey line: Undecaprenyl. Figure adapted from (1).

Supplemental Figure S2



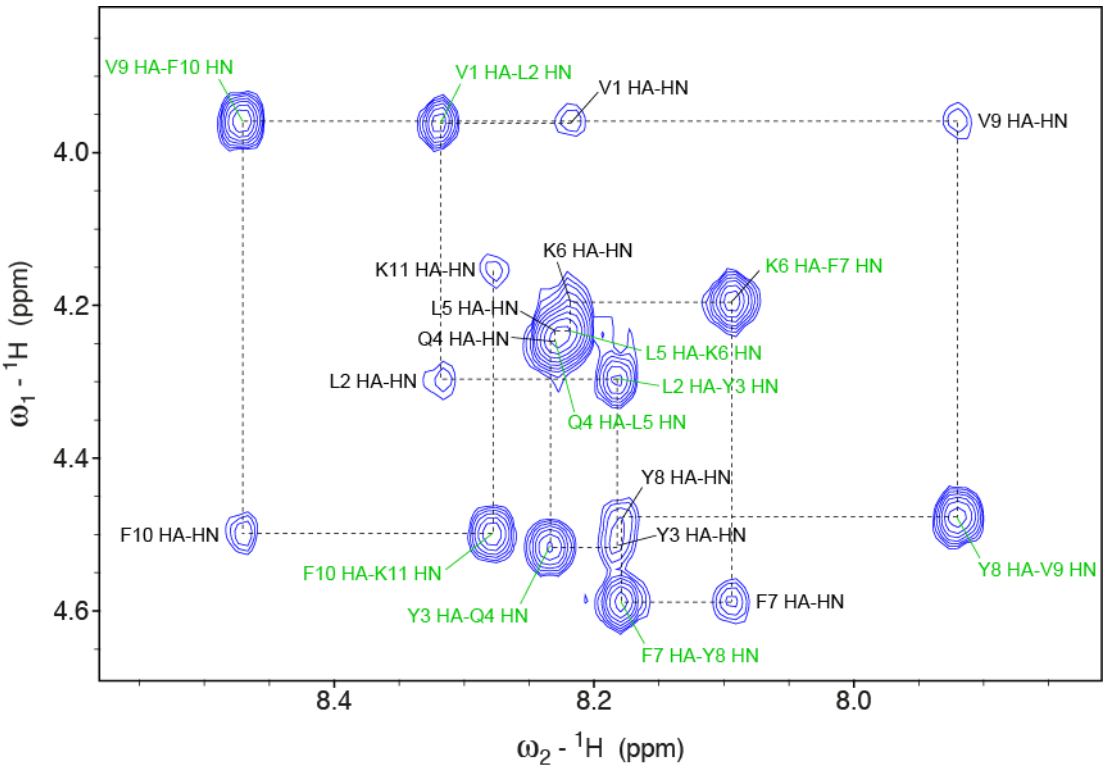
Supplemental Figure S2: Analysis of different EL5 cross-linking mutants. (A) SDS-PAGE of PglB Pos 1 (N321C/L374C) and the internal standard HmuT Q147C labeled with fluorescein-5-maleimide. Left image represents a fluorescence gel scan with 488 nm excitation and 526 nm emission; Right image represents an immunoblot of the same gel detecting His-tagged PglB and HmuT. Bands for PglB and HmuT are indicated. (B) Size exclusion chromatograms of purified and concentrated PglB Pos 1 samples after peptide binding (fluorescence anisotropy) measurements. The black profile shows the oxidized (cross-linked) PglB, whereas the red profile represents the reduced sample in the presence of 80 mM β-Mercaptoethanol. Note that the superimposed chromatograms are not normalized for the amount of protein injected onto the gel filtration column. The analysis is used exclusively to assess monodispersity at elevated concentrations of enzyme and β-Mercaptoethanol. (C) SDS-PAGE of PglB Pos 2 (F311C/F579C). Lane 1: cross-linked (oxidized) PglB; lane 2: reduced PglB in folded state; lane 3: reduced PglB in folded state, concentrated sample; lane 4: reduced PglB in unfolded state. (D) SDS-PAGE of PglB Pos 3 (F308C/K528C). Lane 1: cross-linked (oxidized) PglB; lane 2: reduced PglB in folded state; lane 3: reduced PglB in unfolded state. (C), (D) The mobility shift between oxidized and reduced PglB is indicated.

Supplemental Figure S3



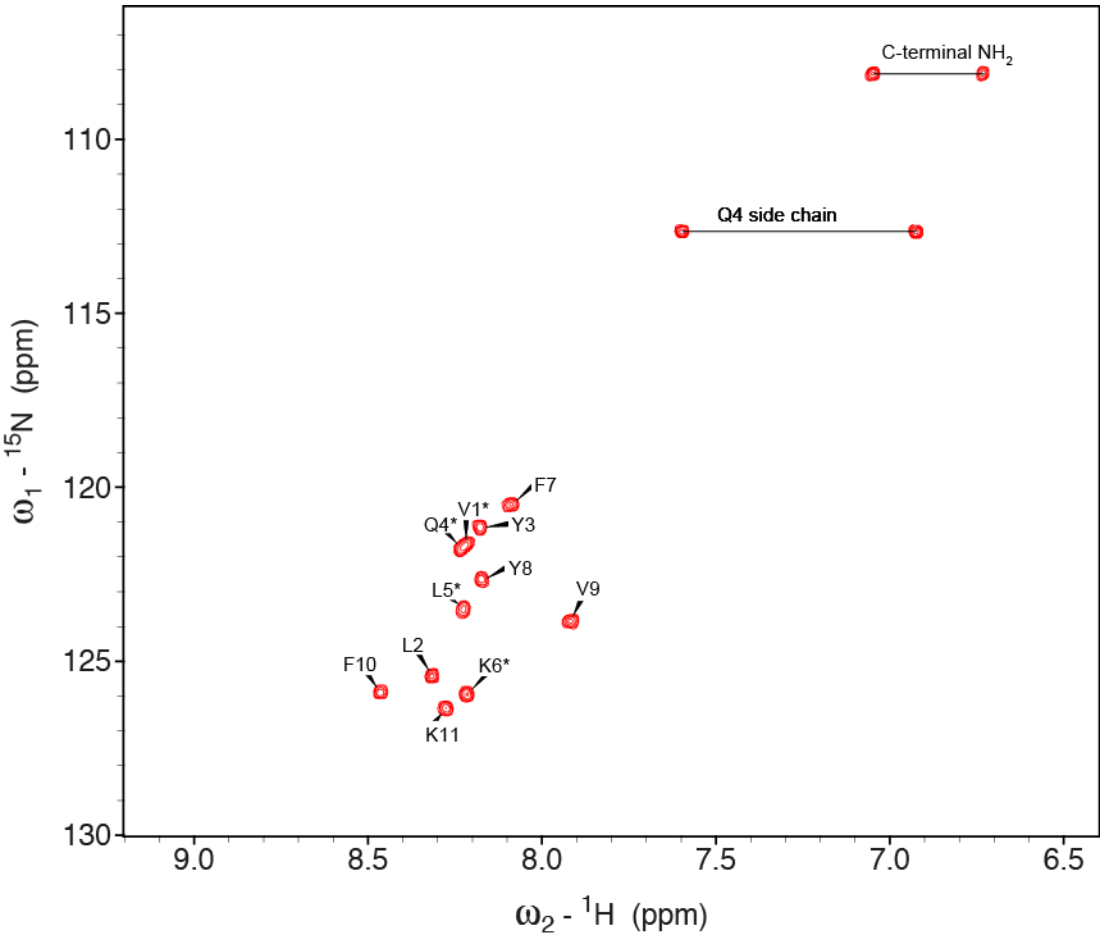
Supplemental Figure S3: Validation of PglB with deletion of TM helices 8 and 9. (A) SDS-PAGE of wt PglB and truncated PglB mutant 7 (ΔTM8/9) with a theoretical mass of 84,362 Da and 79,089 Da, respectively. (B) High-mass MALDI mass spectrum of PglB ΔTM8/9. The spectrum presents the major species of singly charged PglB at m/z = 79,100. The peaks at low molecular weight, e.g. at m/z = 15,300 and 17,100 and the peak close to the PglB (68,900) are likely from impurities or N-terminal degradation that could not be detected by SDS-PAGE.

Supplemental Figure S4



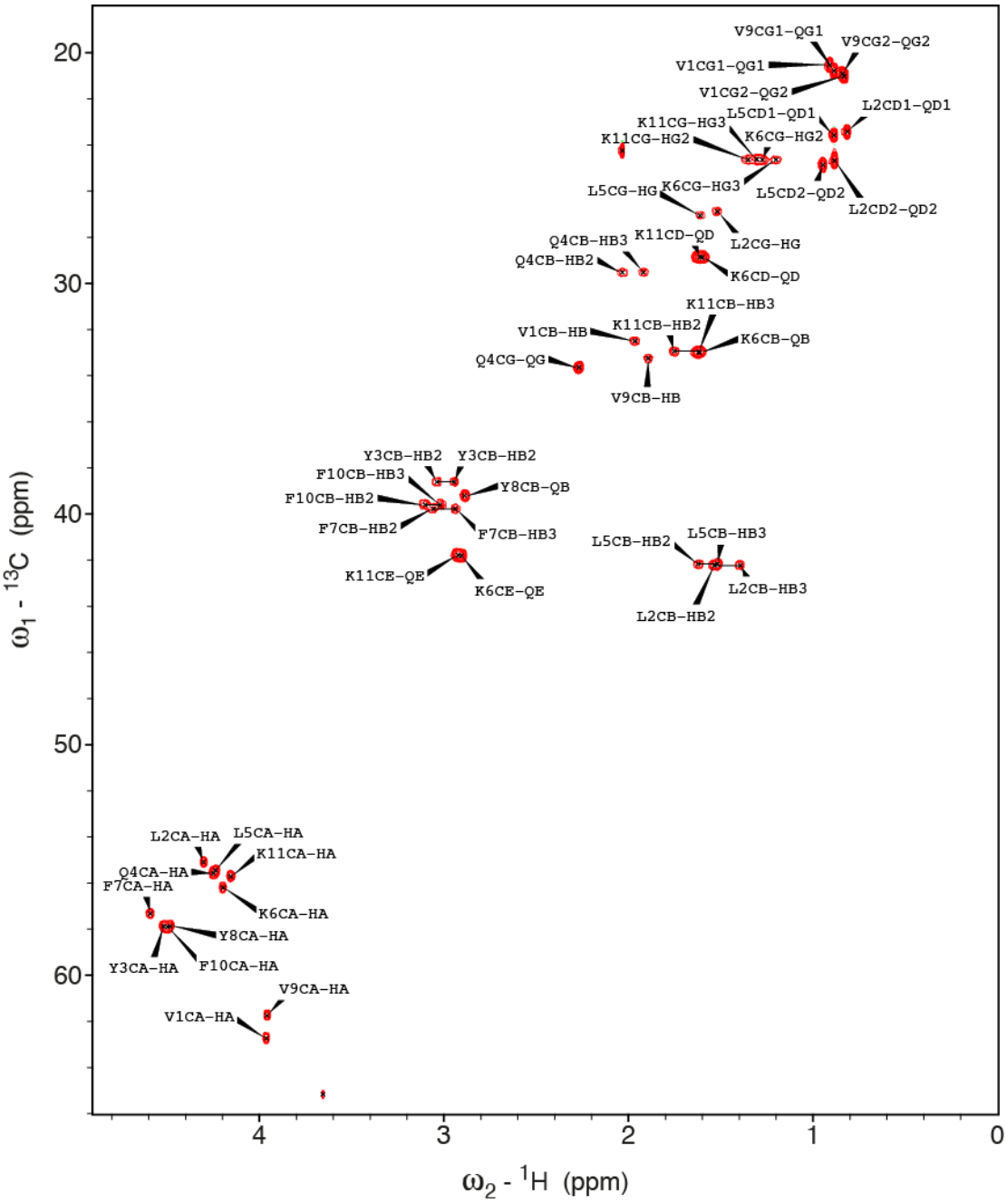
Supplemental Figure S4: 2D ^{1}H-^{1}H NOESY spectrum of peptide EL5-11. Undecapeptide Ac-VLYQLKFYVFK-NH_2 was recorded at 750 MHz and 283 K with 88 transients. The region of the Hα-HN NOE correlations is shown. Only intra-residue (black) and sequential NOE correlations (green) are visible but no long-range NOEs indicating the absence of secondary structure. The sequential Hα-HN assignment walk is indicated by dashed lines.

Supplemental Figure S5



Supplemental Figure S5: Natural abundance 2D ^{15}N-^{1}H HSQC spectrum of peptide EL5-11. Undecapeptide Ac-VLYQLKFYVFK-NH_2 was recorded at 700 MHz and 283 K with 400 transients. Backbone N-HN correlations are labeled with the single letter code and residue number. Two pairs of signals had an identical HN chemical shift so that the ^{15}N chemical shift assignment could not be assigned unambiguously (indicated with an *). The observed small dispersion is indicative of an unstructured peptide.

Supplemental Figure S6



Supplemental Figure S6: Natural abundance 2D ^{13}C-^{1}H HSQC spectrum of peptide EL5-11. Undecapeptide Ac-VLYQLKFYVFK-NH_2 was recorded at 600 MHz and 283 K with 16 transients. All aliphatic side chain signals are labeled.

SUPPLEMENTAL TABLES

Supplemental Table S1: Oligonucleotide primers

Nr.	Sequence
1	5'-TTACATTTAAATGGGGGGGCTCTCTCTC-3'
2	5'-AACACTCGAGCTGATACGAATGGCTAGC-3'
3	5’-CAGGATCTAAGCCACCACTTAAAGCTATGATCATCAAAG-3
4	5’-AAACCATGGAACTACAACAAAATTTCACG-3
5	5’-TTTGATGATCATAGCTTTAAGTGGTGGCTTAGATCCTG-3’
6	5’-TTTGAATTCTCTTTTTAGCCTATAAATTTTTGC-3’
7	5’-AATTCAATTGCCATGGAACTACAACAAAATTTCACGGATAATAATTC-3’
8	5’-TGCCTGCAGTTAAGCGTAATCTGGAACATCGTATGGGTAGAATTCTCTTTTTAGCCTATAAATTTTTGC TCTTGTATC-3’
9	5’-GAATTCATATGAAACTACAACAAAATTTCACGGATAATAATTC-3’
10	5’-CGGACCCTGAAACAGGACTTCCAGAACAGGATCTAAGCCACCACTTAAATG-3’
11	5’-CTGGAAGTCCTGTTTCAGGGTCCGTTCAAAGCTTCTGATGTACAAAATTTAAAAG-3’
12	5’-CTGCAGTTAAGCGTAATCTGGAACATCGTATGGGTATCTTTTTAGCCTATAAATTTTTGCTCTTGTATC-3’
13	5’-AAACCATGGAACTACAACAAAATTTCACG-3’
14	5’-TTTGAATTCTCTTTTTAGCCTATAAATTTTTGC-3’

Supplemental Table S2: Constructed plasmids

Plasmid	Description
pCL67	pET24, expressing glyconegineered GFP-His_6 with insertion of sequence ASGGGGSGGGDQNATGSGGGGSAG after K156 introducing an exposed bacterial glycosylation site (underlined)
DC3	pACYC*pgl::pglJ* with inactivated pglB gene (W458A,D459A) to express a disaccharide LLO structure
Mut1-pMLBAD	pMLBAD, expressing HA-tagged *C.lari* PglB with EL5 replaced by EL5 of *W. succinogenes* ^{280}SGGALSPLWYQLEVYLFRPSVEASAPSLHFYSVVQTIREASTLSLEKLAIRISS^{333}
Mut2-pMLBAD	pMLBAD, expressing HA-tagged *C.lari* PglB with insertion of sequence LEVLFQGP afterA306
Mut3-pMLBAD	pMLBAD, expressing HA-tagged *C.lari* PglB with replacement ^{298}GGSGGSGGS^{306}
Mut4-pMLBAD	pMLBAD, expressing HA-tagged *C.lari* PglB with deletion of aa 298-306
Mut5-pMLBAD	pMLBAD, expressing HA-tagged *C.lari* PglB with replacement ^{287}LEVLFQGP^{294}
Mut5-pBAD	pBAD, expressing His_{10}-tagged *C. lari* PglB K2E with replacement ^{287}LEVLFQGP^{294}
Mut6-pMLBAD	pMLBAD, expressing HA-tagged *C.lari* PglB with insertion of sequence LEVLFQGP after G281
Mut7-pMLBAD	pMLBAD, expressing HA-tagged *C.lari* PglB with deletion of aa 237-280 (TM helices 8,9)
Mut7-pBAD	pBAD, expressing His_{10}-tagged *C. lari* PglB K2E with deletion of aa 237-280 (TM helices 8,9)
Mut8-pMLBAD	pMLBAD, expressing HA-tagged *C.lari* PglB with replacement of sequence ^{297}ASDVQNLKDA^{306} by sequence GGSGGSLEVLFQGPGGSGGS
Mut8-pBAD	pBAD, expressing His_{10}-tagged *C. lari* PglB K2E with replacement of sequence ^{297}ASDVQNLKDA^{306} by sequence GGSGGSLEVLFQGPGGSGGS

SUPPLEMENTAL REFERENCES

1. Lizak, C., Gerber, S., Numao, S., Aebi, M., and Locher, K. P. (2011) X-ray structure of a bacterial oligosaccharyltransferase. *Nature* 474, 350-355