The crystal structure of the major pneumococcal autolysin LytA in complex with a large peptidoglycan fragment reveals the pivotal role of glycans for lytic activity

Supporting Information

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Figure S1. Structures of muropeptides M5P, GM5P and di(GM5P). The tetrasaccharide compound di(GM5P) was used for co-crystallization with LytA.

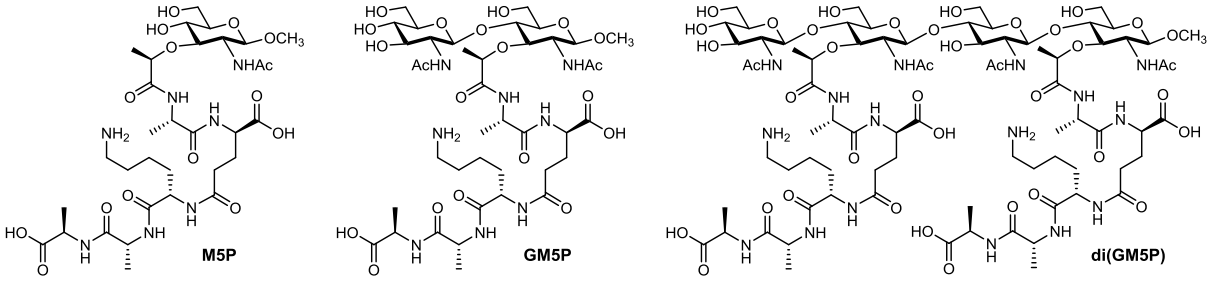


Figure S2. A schematic view of di(GM5P) and LytA^{Ami} interaction reveals a strong binding of the carbohydrate moiety. Most of the interactions between the substrate di(GM5P) and the amidase LytA occur between protein residues and glycan moieties. LytA and substrate components are colored orange and dark-magenta, respectively. Substrate and amidase residues are labeled in blue and green, respectively. Hydrogen bond interactions are displayed as green dashed lines. Van der Waals interactions are presented as orange semicircles. All water molecules were removed for clarity.

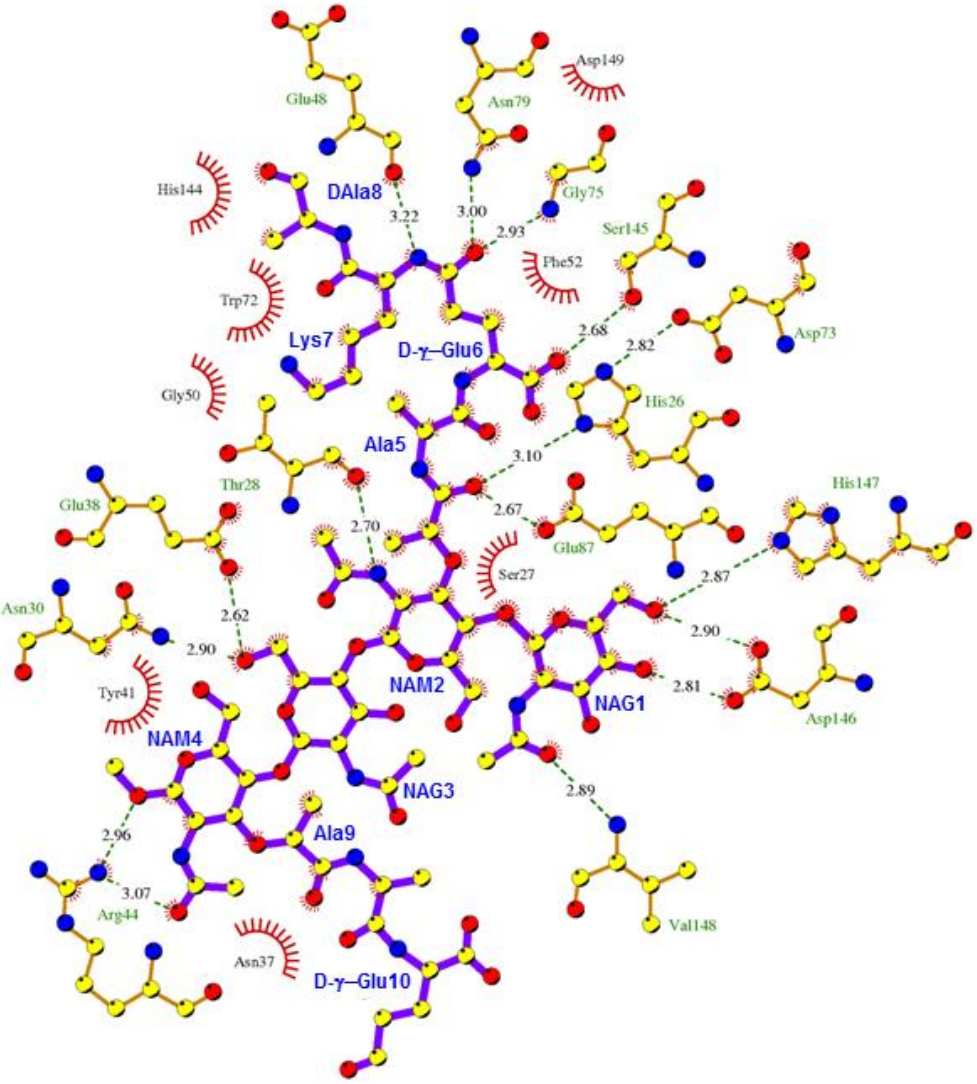


Figure S3. Graphic illustration of the lytic activity of LytA Thr28 mutant proteins in comparison with LytA wt. Treatment of *S. pneumoniae* T4Δ*lytA* cells with recombinant LytA protein (10 μg/ ml) at the time point indicated by the arrow. Optical density at 600 nm measured in 5 min intervals. Curves are based on average values from three independent treatments (error bars omitted for clarity). Treatments correspond to LytA-wt (black spheres), LytA-T28V (white spheres), -T28S (white diamonds), -T28G (black diamonds), T28I (white triangles), -T28A (black triangles), and Mock (buffer) treatment (white squares).

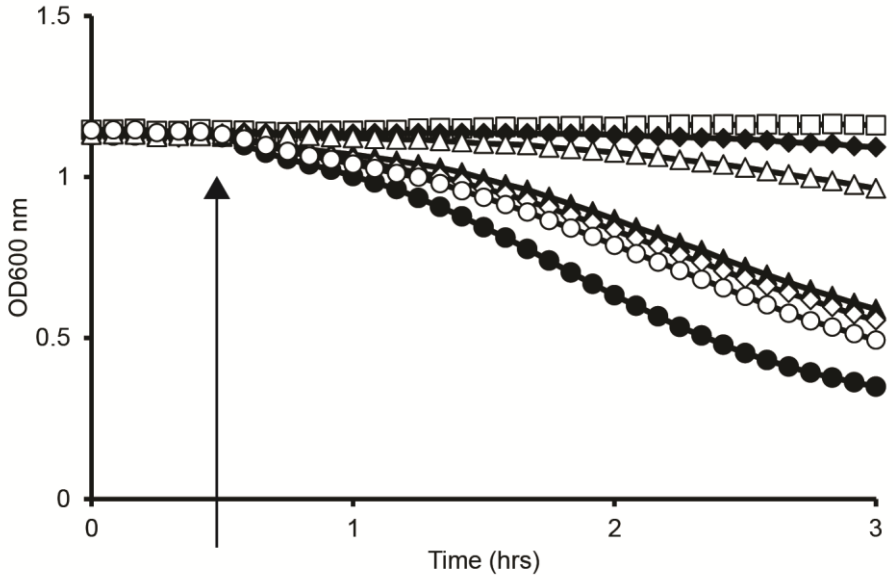


Figure S4. Circular dichroism spectra of the wt LytA and mutants with decreased activity demonstrates native folding of the mutated LytA. Wt LytA (black), T28A (light blue), T28G (red), T28I (green), E38A (orange), R44E (yellow), K45A (gray), D146A (violet), H147A (blue).

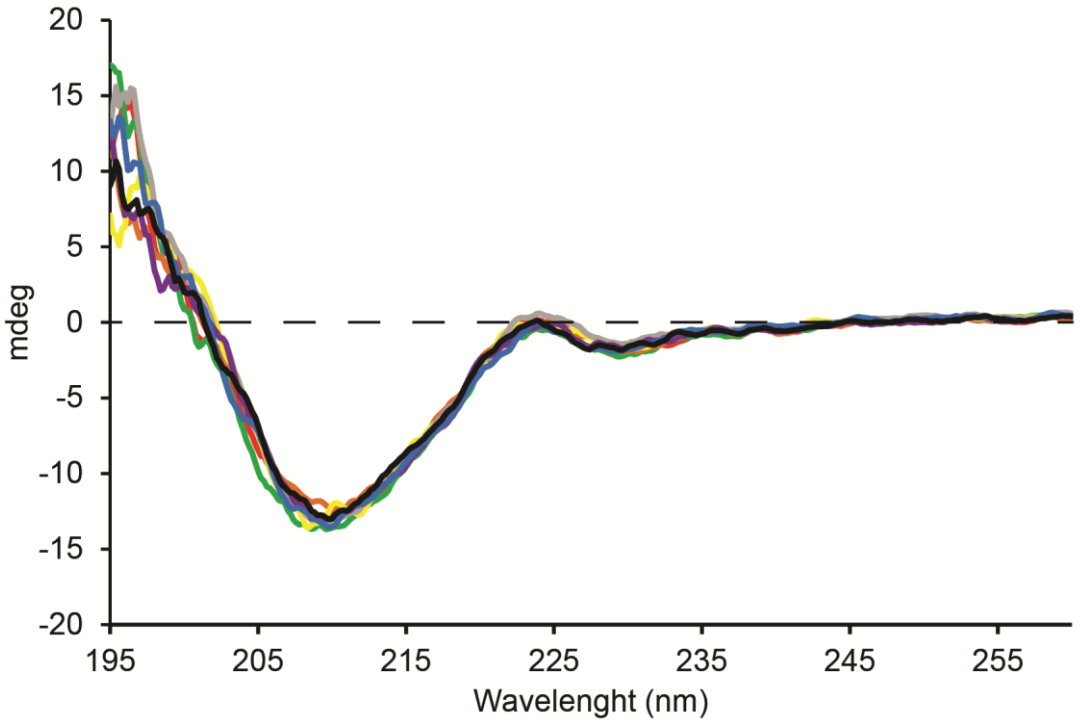
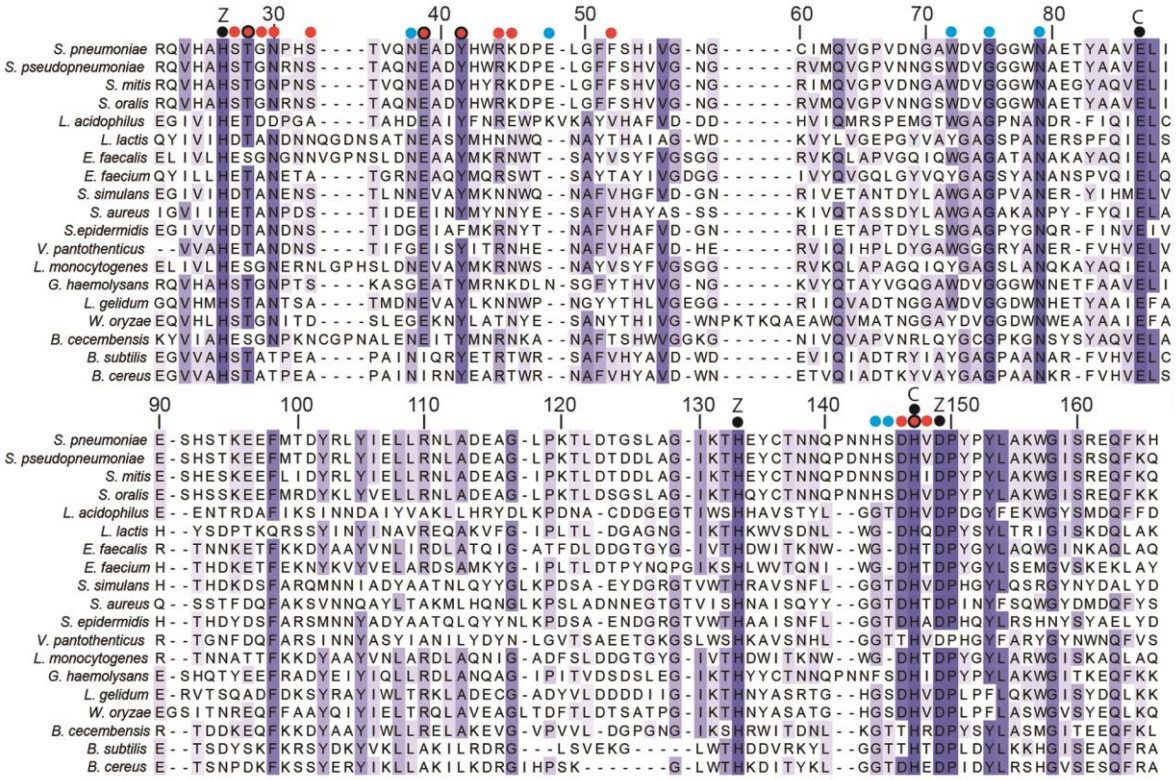


Figure S5. Multisequence alignment of amidases from Firmicute species reveals a common substrate binding motif A multisequence alignment of amidases from a wide range of Firmicute species is presented, displaying conserved residues colored according to sequence conservation (from light to dark blue). The sequence alignment was obtained using Clustal Muscle and the image was created using Jalview. Residue numbers for the *S. pneumoniae* LytA sequence are given above the alignment. Black spheres with 'Z' and 'C' above indicate zinc ligands and catalytic residues, respectively. Red spheres highlight saccharide binding residues. Red spheres with a black frame indicate crucial saccharide-interacting residues required for LytA autolytic activity. Blue spheres indicate peptide-interacting residues. Accession numbers for proteins used in the alignment are *S. pneumoniae* (AAK76005), *S. pseudopneumoniae* (WP_049537327), *S. mitis* (WP_061439982), *S. oralis* (WP_061420134), *Lactobacillus acidophilus* (WP_003548796.1), *Lactococcus lactis* (KHE75992.1), *Enterococcus faecalis* (WP_010709320.1), *Enterococcus faecium* (WP_002314482.1), *Staphylococcus simulans* (WP_061054797.1), *S. aureus* (4KNL_A), *S. epidermidis* (3LAT_A), *Virgibacillus pantothenticus* (WP_050351035.1), *Listeria monocytogenes* (CWW28782), *Gemella haemolysans* (EGF88446.1), *Leuconostoc gelidum* (WP_013231895), *Weissella oryzae* (WP_052348522.1), *Bacillus cecembensis* (WP_057989989.1), *Bacillus subtilis* (CUB50564), and *Bacillus cereus* (WP_016099431.1).


Z 30 40 50 60 70 80 C
S. pneumoniae RQVHAHSTGNPHS----TVQNEADYHWRKDPE-LGFFSHIVG-NG------CIMQVGPVDNGAWDVGGGWNAETYAAVELI
S. pseudopneumoniae RQVHAHSTGNRNS----TAQNEADYHWRKDPE-LGFFSHVVG-NG------RVMQVGPVNNGSWDVGGGWNAETYAAVELI
S. mitis RQVHAHSTGNPNS----TVQNEADYHYRKDPE-LGFFSHVVG-NG------RIMQVGPVDNGAWDVGGGWNAEGYAQVELI
S. oralis RQVHAHSTGNRNS----TAQNEADYHWRKDPE-LGFFSHVVG-NG------RVMQVGPVNNGSWDVGGGWNAETYAAVELI
L. acidophilus EGIVIHETDDPGA----TAHDEAIYFNREWPKVKAYVHAFVD-DD------HVIQMRSPEMGTWGAGPNANDR-FIQIELC
L. lactis QYIVIHDTANDNNQGDNSATNEASYMHNNWQ--NAYTHAIAG-WD------KVYLVGEPGYVAYGAGSPANERSPFQIELS
E. faecalis ELIVLHESGNGNNVGPNSLDNEAAYMKRNWT--SAYVSYFVGSGG------RVKQLAPVGQIQWGAGATANAKAYAQIELA
E. faecium QYILLHETANETA----TGRNEAQYMQRSWT--SAYTAYIVGDGG------IVYQVGQLGYVQYGAGSYANANSPVQIELQ
S. simulans EGIVIHDTANENS----TLNNEVAYMKNNWQ--NAFVHGFVD-GN------RIVETANTDYLAWGAGPVANER-YIHMELV
S. aureus IGVIIHETANPDS----TIDEEINYMYNNYE--SAFVHAYAS-SS------KIVQTASSDYLAWGAGAKANPY-FYQIELA
S.epidermidis EGIVVHDTANDNS----TIDGEIAFMKRNYT--NAFVHAFVD-GN------RIIETAPTDYLSWGAGPYGNQR-FINVEIV
V. pantothenticus --VVAHETANDNS----TIFGEISYITRNHE--NAFVHAFVD-HE------RVIQIHPLDYGAWGGGRYANER-FVHVELV
L. monocytogenes ELIVLHESGNERNLGPHSLDNEVAYMKRNWS--NAYVSYFVGSGG------RVKQLAPAGQIQYGAGSLANQKAYAQIELA
G. haemolysans RQVHAHSTGNPTS----KASGEATYMRNKDLN-SGFYTHVVG-NG------KVYQTAYVGQGAWDVGGGWNNETFAAVELI
L. gelidum GQVHMHSTANTSA----TMDNEVAYLKNNWP--NGYYTHLVGEGG------RIIQVADTNGGAWDVGGDWNHETYAAIEFA
W. oryzae EQVHLHSTGNITD----SLEGEKNYLATNYE--SANYTHIVG-WNPKTKQAEAWQVMATNGGAYDVGGDWNWEAYAAIEFA
B. cecembensis KYVIAHESGNPKNCGPNALENEITYMNRNKA--NAFTSHWVGGKG------NIVQVAPVNRLQYGCGPKGNSYSYAQVELA
B. subtilis EGVVAHSTATPEA----PAINIQRYETRTWR--SAFVHYAVD-WD------EVIQIADTRYIAYGAGPAANAR-FVHVELC
B. cereus EGVVAHSTATPEA----PAINIRNYEARTWR--NAFVHYAVD-WN------ETVQIADTKYVAYGAGPAANKR-FVHVELS
90 100 110 120 130 Z 140 C Z150 160
S. pneumoniae E-SHSTKEEFMTDYRLYIELLRNLADEAG-LPKTLDTGSLAG-IKTHEYCTNNQPNNHSDHVDPYPYLAKWGISREQFKH
S. pseudopneumoniae E-SHSTKEEFMTDYRLYIELLRNLADEAG-LPKTLDTDDLAG-IKTHEYCTNNQPDNHSDHVDPYPYLAKWGISRSQFKQ
S. mitis E-SHESKEEFLIDYRLYIELLRNLADEAG-IPKTLDTDDLAG-IKTHEYCTNNQPDNNSDHIDPYPYLAKWGISREQFKQ
S. oralis E-SHSSKEEFMRDYKLYVELLRNLADEAG-LPKTLDSGSLAG-IKTHQYCTNNQPNNHSDHVDPYPYLAKWGISREQFKK
L. acidophilus E--ENTRDAFIKSINNDAIYVAKLLHRYDLKPDNA-CDDGEGTIWSHHAVSTYL--GGTDHVDPDGYFEKWGYSMDQFFD
L. lactis H--YSDPTKQRSSYINYINAVREQAKVFG-IPLTL-DGAGNG-IKTHKWVSDNL--WG-DHQDPYSYLTRIGISKDQLAK
E. faecalis R--TNNKETFKKDYAAYVNLIRDLATQIG-ATFDLDDGTGYG-IVTHDWITKNW--WG-DHTDPYGYLAQWGINKAQLAQ
E. faecium H--THDKETFEKNYKVYVELARDSAMKYG-IPLTLDTPYNQPGIKSHLWVTQNI--WG-DHTDPYGYLSEMGVSKEKLAY
S. simulans H--THDKDSFARQMNNIADYAATNLQYYGLKPDSA-EYDGRGTVWTHRAVSNFL--GGTDHVDPHGYLQSRGYNYDALYD
S. aureus Q--SSTFDQFAKSVNNQAYLTAKMLHQNGLKPSLADNNEGTGTVISHNAISQYY--GGTDHTDPINYFSQWGYDMDQFYS
S. epidermidis H--THDYDSFARSMNNYADYAATQLQYYNLKPDSA-ENDGRGTVWTHAAISNFL--GGTDHADPHQYLRSHNYSYAELYD
V. pantothenticus R--TGNFDQFARSINNYASYIANILYDYN-LGVTSAEETGKGSLWSHKAVSNHL--GGTTHVDPHGYFARYGYNWNQFVS
L. monocytogenes R--TNNATTFKKDYAAYVNLARDLAQNIG-ADFSLDDGTGYG-IVTHDWITKNW--WG-DHTDPYGYLARWGISKAQLAQ
G. haemolysans E-SHQTYEEFRADYEIYIQLLRDLANQAG-IPITVDSDSLEG-IKTHYYCTNNQPNNFSDHIDPYPYLAKWGITKEQFKK
L. gelidum E-RVTSQADFDKSYRAYIWLTRKLADECG-ADYVLDDDDIIG-IKTHNYASRTG--HGSDHVDPLPFLQKWGISYDQLKK
W. oryzae EGSITNREQFFAAYQIYIELTRQLAVEAGLTDFTLDTSATPG-IKTHNYASATG--HGSDHVDPLPFLASWGVSYEQLKQ
B. cecembensis R--TDDKEQFKKDYAAYIWLLRELAKEVG-VPVVL-DGPGNG-IKSHRWITDNL--KGTTHRDPYSYLASMGITEEQFKL
B. subtilis E--TSDYSKFKRSYDKYVKLLAKILRDRG---LSVEKG-----LWTHDDVRKYL--GGTTHTDPLDYLKKHGISEAQFRA
B. cereus E--TSNPDKFKSSYERYIKLLAKILKDRGIHPSK-------G-LWTHKDITYKL--GGTDHEDPIDYLRSHGVSESQFRA