Supporting Information

Molecular Analysis of an Enigmatic *Streptococcus pneumoniae* Virulence Factor: the Raffinose-Family Oligosaccharide Utilization System

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Running title: Molecular analysis of pneumococcal RFO utilization

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Table S1. Primers used in this study

Primer name	Sequence (5'→3')
Aga-F	CCGCGCGGCAGCCATATGGGAGTTAGGATAGAGAATAATC
Aga-R	GGTGGTGGTGCTCGATCATAGTTTTCTAAAAATATACTG
RafE-F	GCCGCGCGGCAGCCATTCCAACTATGGTAAATCTGCGGATG
RafE-R	GCTCGAATTCGGATCCTAATCCACATCCGCTTTCATCG
GtfA-F	GCCGCGCGGCAGCCAAATGCCAATTCAAAATAAAACCATGTTGATTACCT
GtfA-R	GCTCGAATTCGGATCTCAAAATGATACTTCAACTCCATTCTCAATTACC
Aga D472N-F	GATTACATTAAATGGAATATGAACCGCAATATC
Aga D472N-R	GATATTGCGGTTCATATTCCATTTAATGTAATC

Underlining indicates vector sequence used for In-Fusion® HD cloning (Takara Bio USA, CA).

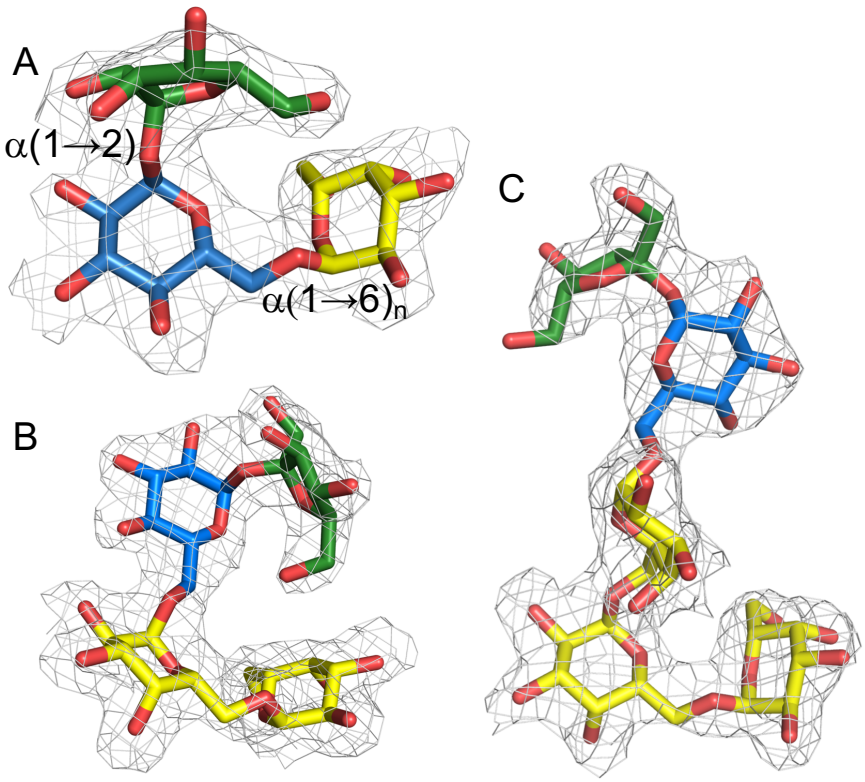


Figure S1. Electron density of RafE bound to raffinose family oligosaccharides. Stick representation of (A) raffinose, (B) stachyose and (C) verbascose within their electron density. The electron densities are shown as a maximum likelihood/σ_a-weighted F_o-F_c map, contoured at 2.0σ (0.19e/Å^3) for raffinose, 2.0σ (0.17e/Å^3) for stachyose and 2.0σ (0.18e/Å^3) for verbascose. Galactose, glucose and fructose are shown in yellow, blue and green, respectively.

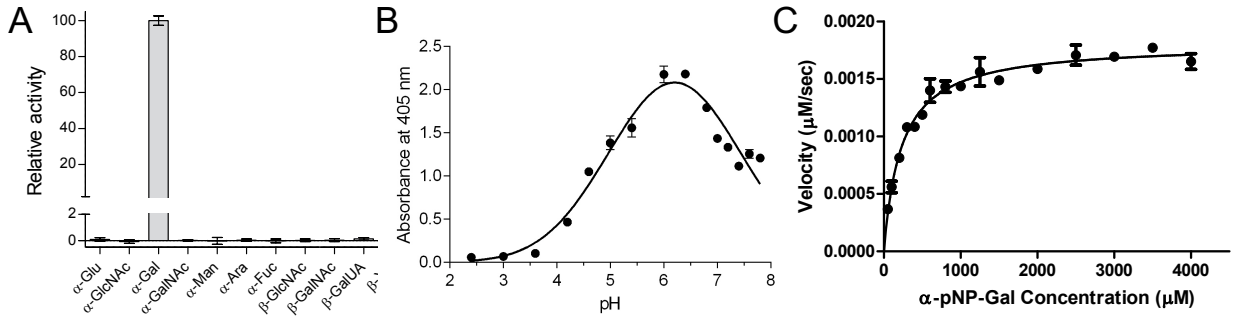
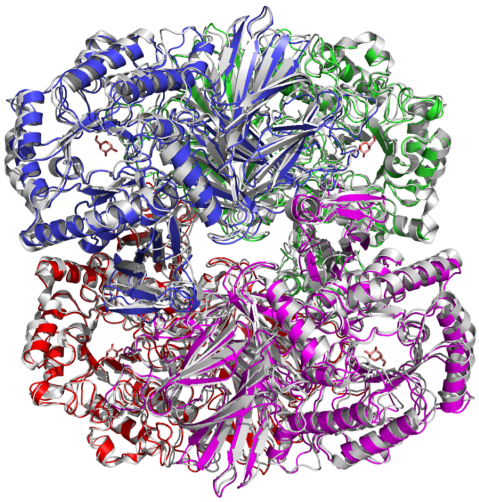


Figure S2. pH profile, Michaelis-Menten plot and activity screen for Aga against pNP substrates. (A) Screening of Aga for activity against a range of pNP substrates. Reactions contained 1.5 mM substrate and 200 nM Aga in 20 mM NaH_2PO_4/K_2HPO_4 at pH 6.5, and were incubated at 37 °C for 1 hour before being stopped by the addition of NaOH. Data are shown relative to α-Gal and are the mean of three replicates; error bars represent the SD. (B) The pH optimum of Aga was determined using McIlvaine buffers and pNP-α-Gal as substrate. Reactions were incubated at 37 °C for 15 minutes and stopped by the addition of NaOH. Data shown are the mean of four replicates. (C) Michaelis-Menten plot for Aga against pNP-α-Gal in 20 mM NaH_2PO_4/K_2HPO_4 at pH 6.5 at 37 °C. Data shown are the mean of three replicates. In both plots, error bars represent the SEM.

A



B

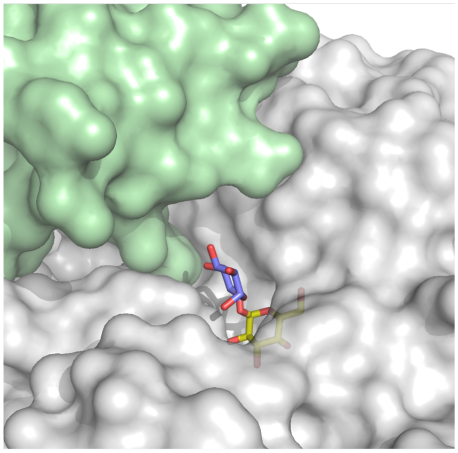


Figure S3. Aga tetramer. (A) Overlay of the Aga tetramer generated by crystallographic symmetry (represented as green, blue, red and purple cartoon) with *Gs*AgaA from *Geobacillus stearothermophilus* (PDB ID 4FNU; gray cartoon). (B) Surface representation of the Aga catalytic site resulting from tetramerization. The catalytic site of the gray monomer harboring melibiose is partially occluded by the green monomer resulting in a narrower catalytic pocket.

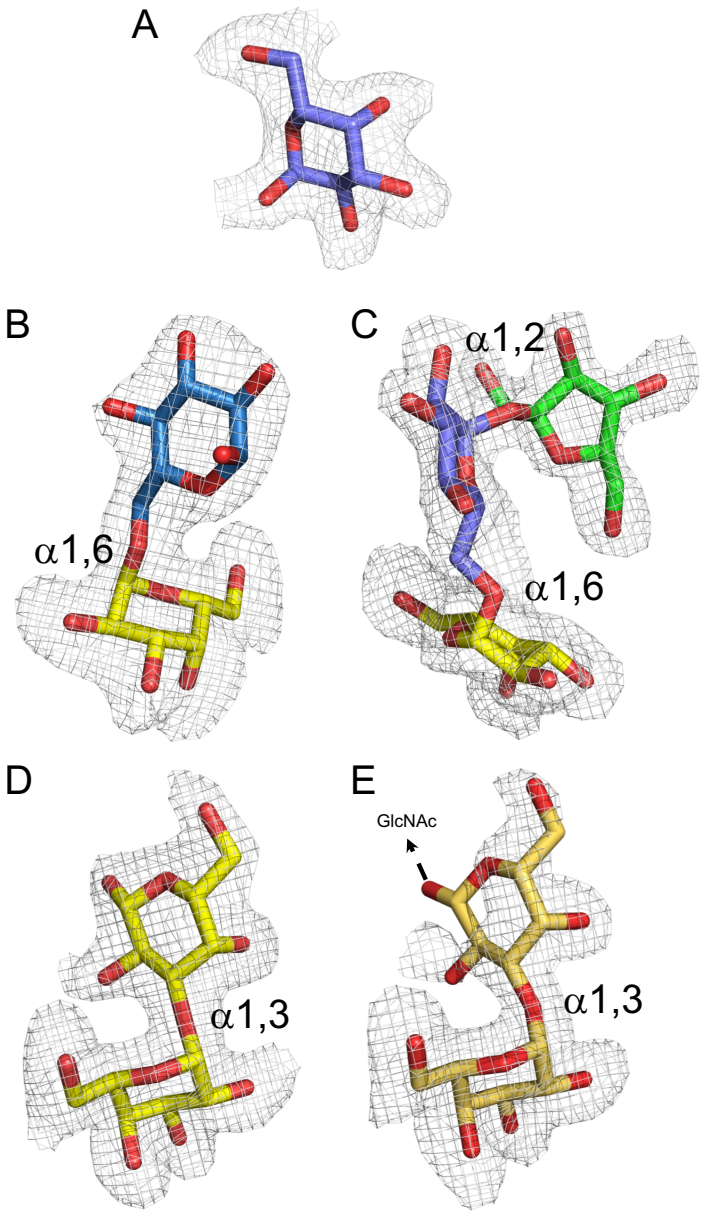


Figure S4. Electron density of Aga-bound product and substrates. Stick representation of (A) galactose, (B) melibiose, (C) raffinose, (D) α-(1→3)-galactobiose and (E) linear type II blood group B trisaccharide within their electron density. The electron densities are shown as a maximum likelihood/σ_a-weighted F_o-F_c map, contoured at 2.0σ (0.19e/Å^3) for galactose, raffinose and α-(1→3)-galactobiose, 2.0σ (0.17e/Å^3) for melibiose and 2.0σ (0.18e/Å^3) for linear type II B trisaccharide. Galactose, glucose and fructose are shown in yellow, blue and green, respectively.

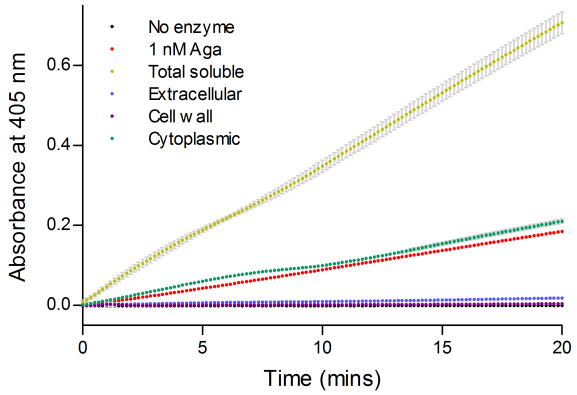


Figure S5. Cellular localization of Aga. α-galactosidase activity of different cellular fractions of *S. pneumoniae* TIGR grown on raffinose against pNP-α-gal. The activity of purified recombinant Aga against pNP-α-gal is shown for comparison. Data shown are the mean of three replicates; error bars, where visible, represent the SEM.