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Supplemental Information

Structural Basis for Regulation and Specificity of Fructooligosaccharide Import in *Streptococcus pneumoniae*

Simone Culurgioni, Gemma Harris, Anirudh K. Singh, Samantha J. King, and Martin A. Walsh

SUPPLEMENTAL INFORMATION

Structural basis for regulation and specificity of fructooligosaccharide import in *Streptococcus pneumoniae*.

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SUPPLEMENTAL FIGURES

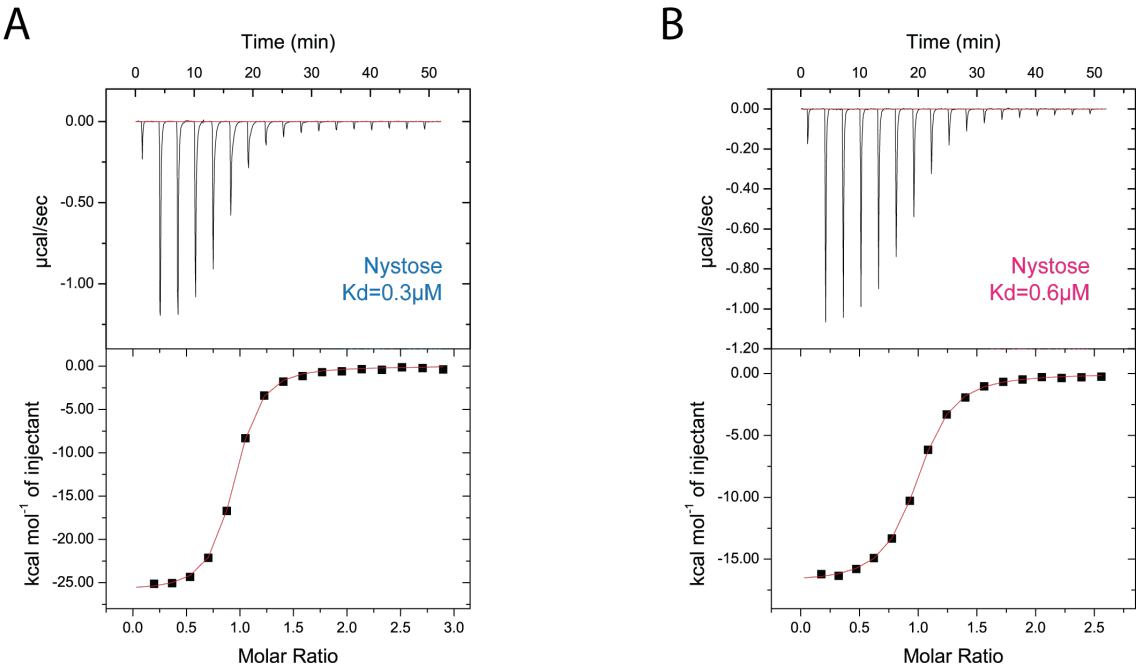

Figure S1 (Related to Figure 1). FusA and SfuA binding to nystose

Measurement of binding affinities of FusA and SfuA towards nystose using Isothermal Titration Calorimetry. All reactions are exothermic and exhibit a 1:1 stoichiometry. (A) FusA binding to nystose. (B) SfuA binding to nystose. K_ds are displayed in the figure.

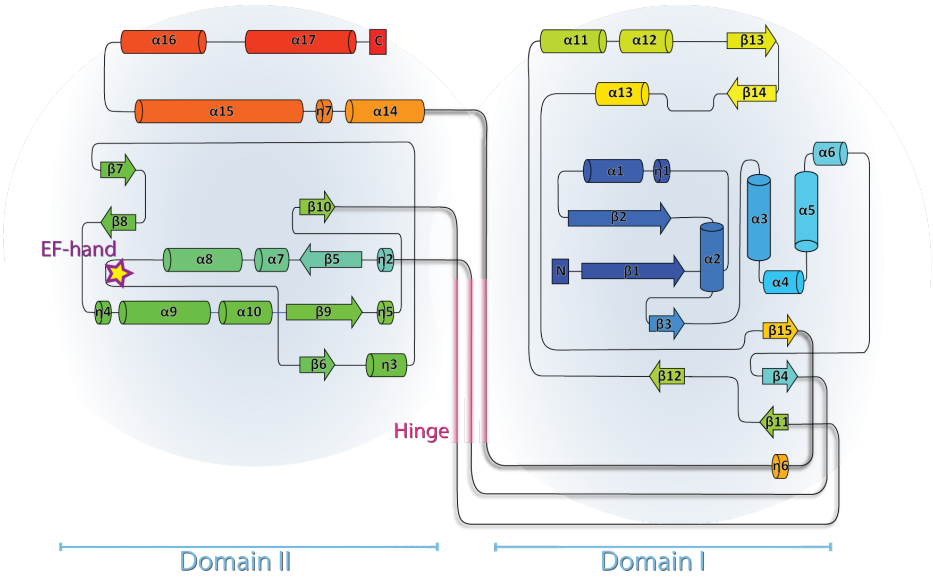

Figure S2 (Related to Figure 3). Identification of a new substrate-binding protein subfamily
Topology diagram of the new subfamily. The secondary structure elements are coloured using rainbow colours (blue to red) starting form the N- to the C-terminus.

SUPPLEMENTAL TABLES

Table S1 – Thermodynamic parameters obtained using ITC (related to Figure 1 and Figure 4)

			Protein Name									
			FusA^{WT}	**FusA$^{D223A-E224A}$**	**FusA^{E167A}**	**FusA^{H177A}**	**FusA^{W314A}**	**FusA^{N318A}**	**FusA^{W384A}**	**FusA^{R419A}**	**FusA^{E423A}**	**SFuA^{WT}**
FOS	**GF1 (0.3 mM)**	[Protein] (μM)	20.0									24.0
		Kd (μM)	NB									NB
		n	-									-
		ΔH (kcal mol^{-1})	-									-
		ΔS (cal mol^{-1} K^{-1})	-									-
	GF2	[Protein] (μM)	20.0	17.0	16.5	16.0	20.2	300.0	63.1	15.8	20.0	24.0
		[GF2] (mM)	0.3	0.3	0.3	0.3	0.3	5.4	25.0	0.3	0.3	0.3
		Kd (μM)	2.2 (±6.9%)	1.9 (±3.4%)	NB	5.5 (±8.3%)[a]	NB	346.0 (±8.5%)[a]	4100.0 (±2.9%)[a]	NB	14.4 (±1.6%)[a]	0.1 (±7.7%)
		n	0.9 (±1.3%)	0.8 (±0.7%)	-	0.4 (±9.2%)[a]	-	0.8 (±10.3%)[a]	1.0[a,b]	-	1.0[a,b]	0.9 (±0.3%)
		ΔH (kcal mol^{-1})	-26.6 (±1.7%)	-26.2 (±0.9%)	-	-32.9 (±10.5%)[a]	-	-20.8 (±12.3%)[a]	-15.1 (±19%)[a]	-	-16.7 (±5.4%)[a]	-19.6 (±0.5%)
		ΔS (cal mol^{-1} K^{-1})	-63.3	-61.5	-	-86.4[a]	-	-53.8	-39.7[a]	-	-33.9[a]	-33.4
	GF3	[Protein] (μM)	22.0	20.0	16.5	18.6	20.2	300.0	63.1	15.8	17.5	24.0
		[GF3] (mM)	0.3	0.3	0.3	0.3	0.3	3.2	25.0	0.3	0.3	0.3
		Kd (μM)	0.3 (±4.7%)	0.4 (±7.7%)	NB	0.8 (±6.7%)[a]	NB	126.6 (±6.4%)	1900.0 (±1.0%)[a]	NB	5.9 (±6.5%)[a]	0.6 (±3.8%)
		n	0.9 (±0.3%)	0.9 (±0.6%)	-	0.4 (±1.3%)[a]	-	1.0 (±2.0%)	1.0[a,b]	-	0.9 (±2.6%)[a]	0.9 (±0.3%)
		ΔH (kcal mol^{-1})	-26.0 (±0.5%)	-25.6 (±0.9%)	-	-26.9 (±1.7%)[a]	-	-17.1 (±3.1%)	-19.4 (±0.6%)[a]	-	-22.8 (±3.4%)[a]	-16.9 (±0.4%)
		ΔS (cal mol^{-1} K^{-1})	-57.6	-56.7	-	-62.2[a]	-	-39.5	-53.1[a]	-	-52.4[a]	-28.2
	GF4	[Protein] (μM)	22.0	20.0	16.5	18.6	20.2	300.0	63.1	15.8	17.5	24.0
		[GF4] (mM)	0.3	0.3	0.3	0.3	0.3	5.4	25.0	0.3	0.3	0.3
		Kd (μM)	0.4 (±5.8%)	0.3 (±12.4%)	NB	1.0 (±5.4%)[a]	NB	222.2 (±4.2%)	2300.0 (±16.1%)[a]	NB	3.7 (±7.3%)[a]	1.2 (±4.4%)
		n	0.9 (±0.5%)	0.9 (±0.8%)	-	0.4 (±1.2%)[a]	-	1.0 (±2.6%)	1.0[a,b]	-	0.8 (±2.2%)[a]	0.9 (±0.5%)
		ΔH (kcal mol^{-1})	-25.6 (±0.7%)	-23.2 (±1.2%)	-	-26.4 (±1.6%)[a]	-	-19.1 (±3.4%)	-20.0 (±1.0%)[a]	-	-22.5 (±2.9%)[a]	-15.8 (±0.7%)
		ΔS (cal mol^{-1} K^{-1})	-56.9	-47.8	-	-60.8[a]	-	-47.3	-54.7[a]	-	-50.5[a]	-26.0
	GFn (0.3 mM)	[Protein] (μM)	43.5	36.7								54.7
		Kd (μM)	1.8 (±1.8%)[c]	1.5 (±3.7%)[c]								9.9 (±7.3%)[a,c]
		n	0.5 (±0.3%)[c]	0.6 (±0.5%)[c]								0.6 (±1.8%)[a,c]
		ΔH (kcal mol^{-1})	-42.0 (±0.3%)[c]	-39.3 (±0.7%)[c]								-21.8 (±2.9%)[a,c]
		ΔS (cal mol^{-1} K^{-1})	-114.0[c]	-105.0[c]								-50.5[a,c]

NB – No binding

[a] Estimated values because of insufficient curvature to accurately determine the binding parameters

[b] Stoichiometry fixed to 1.0 during data fitting

[c] Estimated values dependent on estimated inulin concentration

Table S2 (Related to Figure 4). Hydrogen bonding interactions between kestose (GF2), nystose (GF3), fructofuranosyl-nystose (GF4) and FusA residues.

FOS atom	Protein atom	FusA:GF2 Distance (Å)	FusA:GF3 Distance (Å)	FusA:GF4 Distance (Å)
F_1 [O2]	Glu167[OE2]	2,46	2,64	2,76
F_1 [O3]	Glu167[OE1]	2,61	2,58	2,74
F_1 [O3]	Trp384[NE1]	2,96	2,89	2,93
F_1 [O6]	Trp314[NE1]	2,95	2,93	3,03
F_1 [O1]	Arg419[NH1]	2,86	2,95	3,10
F_1 [O3]	Arg419[NH1]	2,93	2,90	3,11
F_1 [O3]	Arg419[NH2]	3,37	3,35	3,30
F_2 [O1]	Arg419[NH1]	3,05	2,89	2,92
F_2 [O3]	Arg419[NH1]	2,74	2,74	2,98
F_2 [O4]	Asn318[ND2]	2,86	2,85	2,88
F_3 [O3]*	Glu423[OE1]	2,76	2,75	2,74
F_3 [O4]*	Glu423[OE2]	2,67	2,60	2,68

* F_3 [O3] and F_3 [O4] are G_1[O3] and G_1[O4] respectively in G

Table S3 (Related to Figure 5) Growth characteristics of TIGR4 strains grown on nystose[a]

Strain	Maximum OD_{600}	Generation time (h)[b]
TIGR4 Sm^r	0.314-0.458	2.03-2.67
Δ*susTTX*	<0.02	-
E167A	<0.02	-
H177A	0.054-0.152	6.92-9.49
D223A, E224A	<0.02	-
W314A	<0.02	-
N318A	0.109-0.198	4.72-14.51
W384A	0.162-0.239	2.64-5.39
R419A	<0.02	-
E423A	0.272-0.308	2.48-3.89

[a] Ranges represent values from three independent experiments
[b] Calculated during maximal growth rate and not calculated for the strains attaining a maximum OD_{600} <0.02

SUPPLEMENTAL MOVIE

Supplementary Movie 1 (Related to Figure 2). FusA conformational change upon FOS binding.
Morphing between the open and closed conformation of FusA (orange ribbons) induced by the binding of nystose (green sticks).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

ITC (Isothermal Titration Calorimetry)

The proteins were prepared by dialysis against the ITC buffer overnight and subsequent dilution using the ITC buffer. The concentration of proteins was determined using UV spectroscopy at 280 nm. The sugars were resuspended directly into the ITC buffer used for dialysis. Fructofuranosyl-nystose, nystose, kestose and sucrose concentrations were calculated on the basis of the weight of lyophilised material. The concentration of inulin was estimated on the mass of lyophilised material, assuming an average molecular weight of 6017.2 Da.
Isothermal titration calorimetric measurements were carried out using a MicroCal iTC200 microcalorimeter (Malvern Instruments UK) at 25°C. 200 µL of the protein solution was placed in the cell and 40 µL of the respective FOS, at 12.5-400 times the protein concentration, in the syringe. Sixteen 2.4 µL injections, or nineteen 2.0 µL injections, were performed at an injection speed of 0.5 µL/sec, with a pre-injection of 0.5 µL, a

three-minute interval between injections and a stirrer speed of 1000 rpm. To establish the heat of dilution, a control experiment was performed where the syringe solution was injected into the ITC buffer using identical experimental conditions. This was then subtracted from the main experiment.
Data were analysed using MicroCal Origin software (version 7) fitting to a single site binding model. The binding enthalpy (ΔH), association constant (Ka), and the binding stoichiometry (n) were permitted to float (except where noted) during the least-squares minimization process and taken as the best-fit values, with the dissociation constant (Kd) and change in entropy (ΔS) derived subsequently (Table S1).

Generation of mutants for growth assays.
The plasmids generated to express mutant substrate-binding proteins for binding studies were used to introduce the same mutations into *S. pneumoniae* strain TIGR4 using the Janus cassette selection system (Sung et al., 2001). The presence of the intended mutations and the absence of spurious mutations were confirmed by PCR and sequencing. This method requires two rounds of transformation. The first introduced a Janus cassette encoding kanamycin resistance and streptomycin sensitivity (*rpsL*-) into *fusA* (SP_1796) of streptomycin resistant TIGR4 (TIGR4 Sm^r). To generate the Janus construct Phusion (NEB) was used to amplify an inverse PCR product of p1796 E167A (Inv1; ATGAGTAAATGTGCCCATCA and Inv 2; GCTATCCTAGATTCATACTAT) and the Janus cassette [primers J.F and J.R (Marion et al., 2011a; Marion et al., 2011b)]. The Janus amplification product was phosphorylated using T4 Polynucleotide Kinase (Thermo Scientific) and ligated to the inverse PCR product using T4 Ligase (Thermo Scientific). Confirmed constructs were transformed into TIGR4 Sm^r and intermediates containing the Janus construct selected on kanamycin. Following confirmation by PCR (fusAF; CAGCTTGCCATTGATTGTTATG and fusAR; ATCTAACTCGAAGTAATCTGGG) the intermediate was transformed with the expression plasmids encoding each individual point mutation and transformants were selected on streptomycin.

SUPPLEMENTAL REFERENCES

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