

S2 Appendix: Supplemental Tables S1 to S6

Supplemental Table S1: Crystallographic data and refinement statistics

	DacA_{CD} (apo)	DacA_{CD} (ApCpp)	DacA_{CD}-C	GlmM
Data collection				
Space group	P 21 21 21	P 21 21 21	P 21 21 21	P 21 21 21
Cell dimensions				
<i>a</i> , <i>b</i> , <i>c</i> (Å)	54.8 68.19 76.80	44.09 80.42 96.19	44.86 79.77 98.81	88.65 93.88 156.31
α , β , γ (°)	90.00 90.00 90.00	90.00 90.00 90.00	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	68.19 (1.7)*	61.70 (2.6)	98.81 (2.77)	93.88 (3.0)
<i>R</i> _{merge}	0.077 (0.627)	0.116 (2.115)	0.084 (1.147)	0.158 (1.868)
<i>CC</i> _(1/2)	0.999 (0.968)	0.997 (0.825)	0.998 (0.892)	0.998 (0.842)
<i>I</i> / $\sigma(I)$	13.9 (2.0)	10.2 (2.0)	14.2 (2.2)	8.4 (1.8)
Completeness (%)	99.9 (99.0)	99.3 (99.2)	99.9 (99.9)	100.0 (100.0)
Redundancy	12.2 (10.0)	12.2 (12.3)	12.0 (12.2)	12.5 (12.5)
Refinement				
Resolution (Å)	1.7	2.6	2.77	3.0
No. reflections	395176	133313	114145	334706
Unique reflections	32376	10930	9512	26871
<i>R</i> _{work} / <i>R</i> _{free}	0.1735/0.2004	0.2313/0.2576	0.2101/0.2312	0.2146/0.2547
R.m.s. deviations				
Bond lengths (Å)	0.006	0.004	0.008	0.004
Bond angles (°)	0.962	0.731	1.199	0.789

* refers to the highest resolution shell

Supplemental Table S2: Theoretical and calculated experimental masses of DacA_{CD} and GlmM species

Species	Theoretical Mass (Da)	Calculated Mass (Da)
Tetramer	138,210	139,003 ± 10.41
GlmM - dimer	99,462	99,983 ± 19.86
GlmM – monomer	49,731	50,302 ± 22.99
DacA – dimer	38,748	39,232 ± 20.91
DacA – monomer	19,374	19,697 ± 59.15
Octamer	276,420	277,896 ± 111.24

Supplemental Table S3: SAXS data statistics

	DacA _{CD}	GlmM	DacA _{CD} /GlmM
Beamline	B21	B21	B21
Wavelength (Å)	1.0	1.0	1.0
q-range (Å ⁻¹)	0.0032-0.38	0.0032-0.38	0.0032-0.38
I(0) (cm ⁻¹)	0.048 ± 4.4x10 ⁻⁵	0.09 ± 6.8x10 ⁻⁵	0.12 ± 1.2x10 ⁻⁴
R _g (Å)	26.3 ± 0.27	36.8 ± 0.26	38.7 ± 0.10
D _{max} (Å)	85	121.5	125
Guinier region	0.2280-1.2928	0.2928-1.2840	0.2049-1.2919
Porod Volume (Å ³)	64500	128000	204000
MW Estimate (kDa)	40.2	94.2	130.9
Number of atoms (Dammif)	1895	2491	2235

Supplemental Table S4: Collision Cross section (CCS) parameters

	Theoretical CCS (Å ²)*		Charge State	Calculated ^{TW} CCS _{N₂→He₂} (Å ²)
	IMPACT	EM $\cap$ IM		
Tetramer	-	6790	21+	6690
			22+	6610
			23+	6460
			24+	6340
GlmM - Dimer	6890	5550	18+	4750
			19+	5220
			20+	5310
			21+	5420
GlmM - Monomer	3820	-	13+	3210
			14+	3350
DacA - Dimer	2870	3200	12+	2890
			13+	3080
DacA-Monomer	1620	-	8+	1810
			9+	1970

*IMPACT values refer to crystal structure data. EM $\cap$ IM values are based on SAXS structural models.

Supplemental Table S5: Bacterial strains used in this study

Unique ID	Relevant features	Reference
<i>Escherichia coli</i> strains		
ANG127	XL1-Blue- Cloning strain; TetR –	Stratagene
ANG191	Protein expression strain –BL21(DE3)	Novagen
ANG1858	XL1-Blue pET28b-His- <i>dacA_{CD}</i> ; KanR	This study
ANG1865	BL21 (DE3) pET28b-His- <i>dacA_{CD}</i> ; KanR	This study
ANG3994	XL1-Blue pET28b- <i>glmM</i> -His; KanR	This study
ANG3997	BL21 (DE3) pET28b- <i>glmM</i> -His; KanR	This study
ANG4319	XL1-Blue pET28b-His- <i>dacA_{CD}</i> -K; KanR	This study
ANG4320	BL21 (DE3) pET28b-His- <i>dacA_{CD}</i> -K; KanR	This study
ANG4321	XL1-Blue pET28b-His- <i>dacA_{CD}</i> -C; KanR	This study
ANG4322	BL21 (DE3) pET28b-His- <i>dacA_{CD}</i> -C; KanR	This study
ANG4066	XL1-Blue pBAD33; CamR	[1]
ANG4120	XL1-Blue pBAD33- <i>dacA</i> ; CamR	This study
ANG4121	XL1-Blue pBAD33- <i>dacA-ybbR</i> ; CamR	This study
ANG4122	XL1-Blue pBAD33- <i>dacA-ybbR-glmM</i> ; CamR	This study
ANG4263	XL1-Blue pBAD33- <i>dacA-no-ybbR-glmM</i> ; CamR	This study
ANG5168	XL1-Blue pTrcHis60	[2]
ANG5169	XL1-Blue pMLJ4 (GlmM-His <i>E. coli</i>), AmpR	[3]
ANG5171	XL1-Blue pMLJ11 (GlmM-His <i>S. aureus</i>), AmpR	Mengin-Lecreulx
ANG5172	XL1-Blue pMLD137 (GlmM-His <i>P. aeruginosa</i>), AmpR	[4]
ANG5174	XL1-Blue pBAD33- <i>dacA</i> , pTrcHis60; CamR, AmpR	This study
ANG5175	XL1-Blue pBAD33- <i>dacA</i> pMLJ4 (GlmM-His <i>E. coli</i>), CamR, AmpR	This study
ANG5177	XL1-Blue pBAD33- <i>dacA</i> pMLJ11 (GlmM-His <i>S. aureus</i>), CamR, AmpR	This study
ANG5178	XL1-Blue pBAD33- <i>dacA</i> pMLD137 (GlmM-His <i>P. aeruginosa</i>), CamR, AmpR	This study
<i>Staphylococcus aureus</i> strains		
AH1263	LAC* – ANG1575	[5]

Antibiotics were used at the following concentrations: Tetracycline (TetR) 10 µg/ml, Kanamycin (KanR) 30 µg/ml, Ampicillin (AmpR) 100 µg/ml, Chloramphenicol (CamR) 20 µg/ml

Supplemental Table S6: Primers used in this study

Number	Name	Sequence
ANG928	F-KpnI-02407	TTTGGTACCTATTACCCGGAGGAGATG
ANG1135	5-NheI-101AA-SAV2163	CTAGCTAGCTTTTTTAAAACGCTATACTTCTAATACGTATAG
ANG1137	3-EcoRI-SAV2163	CGGAATTCCTATTTTCACACCTTTCTTTTGAAAGCGTG
ANG1244	5-seq-pBAD18	GCACGGCGTCACACTTTGCTATGCC
ANG1245	3-seq-pBAD18	GGCAAATTCTGTTTTATCAGACCGC
ANG2342	5-NcoI-GlmM	CACGCCATGGGAAAATATTTTGGTACAGACGGAGTAAGAGG
ANG2343	3-XhoI-GlmM	CCGCTCGAGGCCGCTGCTGCCGCGCGGCACCAGTTTATCTAATC CCATTTTATCTTGAACC
ANG2475	R_HindIII_dacA	GCGCAAGCTTTTATTTTCACACCTTTCTTTTGAAAGCG
ANG2476	R_HindIII_dacAybbR	GCGCAAGCTTTTATTTTACATTTATATAAGCCTTCG
ANG2477	R_HindIII_dacAybbR glmM	GCGCAAGCTTTTATTTATCTAATCCCATTTTATCTTG
ANG2597	front-YbbR-no start	CATTTACTTTCTAGCTAATTATTTTCACACCTTTCTTTTGAAAGCG
ANG2598	back-YbbR-no start	GTGAAATAATTAGCTAGAAAGTAAATGGGGCTTG
ANG2616	3-front-dacA- N166K/T172K	GGTTTGTTAGGTATAAAGACTTTAATTAAAAGTTCTTGCG
ANG2617	5-back-dacA- N166K/T172K	ATTAAAGTCTTTATACCTAACAAACCTTTACATGATGGTGC
ANG2618	3-front-dacA- N166C/T172C	GGGCAGTTAGGTATAAAGACGCAAATTAAAAGTTCTTGCGAAA TATTG
ANG2619	5-back-dacA- N166C/T172C	TTTGCGTCTTTATACCTAACTGCCCTTTACATGATGGTGCAATG

Restriction sites in primer sequences are underlined

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3. Jolly L, Ferrari P, Blanot D, Van Heijenoort J, Fassy F, Mengin-Lecreulx D. Reaction mechanism of phosphoglucosamine mutase from *Escherichia coli*. *European journal of biochemistry.* 1999;262(1):202-10. Epub 1999/05/07. PubMed PMID: 10231382.
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5. Boles BR, Thoendel M, Roth AJ, Horswill AR. Identification of genes involved in polysaccharide-independent *Staphylococcus aureus* biofilm formation. *PLoS One.* 2010;5(4):e10146. Epub 2010/04/27. doi: 10.1371/journal.pone.0010146. PubMed PMID: 20418950.