Supplementary Data Table 1. **Mass spectrometric identification of GltAB as the interaction partner of GudB.** Top hits in the proteomics analysis of the eluates from the immunoprecipitation of GudB from *B. subtilis* cell lysate grown in glucose-ammonia and in histidine, and in the presence and absence of the crosslinker DSG. Both GltA and GltB are highly represented in the glucose-ammonia sample compared to histidine, both in untreated cells and in cells treated with crosslinker (-/+ DSG; the results are shown in this order, *e.g.*, 103 identified peptides without DSG and 124 identified peptides with DSG).

	Protein ID	Protein	# of peptides [-/+ DSG]	Ratio Glucose: Histidine [/+ DSG]	Sequence coverage [%] [/+ DSG]	Mol. weight [kDa]
Glucose -Ammonia	P39812	**GltA**	103/124	21.69/167.61	73.6/85	**168.77**
	P50735	GudB	30/42	0.56/0.37	78/79.6	47.34
	O34399	**GltB**	27/36	15.86/94.28	61.3/73.6	**54.862**
Histidine	P50735	GudB	30/42	0.56/0.37	78/79.6	47.34
	P28598	GroL	41/38	0.11/0.31	84.9/77.2	57.424
	P28599	GroS	9/13	0.18/0.99	68.1/89.4	10.176

Supplmentary Data Table 2: Steady state kinetic parameters of GudB, GltAB and the GudB-GltAB complex. Reactions were initiated by the addition of enzyme (5 µg of recombinant GudB, or 2.5 µg of the GudB-GltAB complex) and their progress was monitored by absorbance at 340 nm. The concentration of the fixed substrates were 4 mM of NAD^+ for the dehydrogenase reaction, and 2 mM AKG, 5 mM glutamine, and 200 µM NADPH, for the glutamate synthase reaction. The reactions were carried out in 50 mM HEPES pH 7.9 at 25 °C. H is the Hills coefficient (measured for standalone GudB, and also for the GudB-GltAB complex, with glutamate as substrate). * The K_M values for GudB and $GudB_{complex}$ were derived from fitting the data to an allosteric sigmoidal model. All data are presented as mean ± S.D of two independent measurements.

Enzyme/ Varied substrate	K_M	H	k_{cat} (s^{-1})	k_{cat} / K_M ($mM^{-1}\,s^{-1}$)
GudB				
Glutamate	7.3 ± 0.7 mM*	0.59 ± 0.06	9.8	1.34
$GudB_{complex}$				
Glutamate	138 ± 33 mM*	1.3 ± 0.07	11	0.08
GltAB				
Glutamine	76.6 ± 6.7 µM		65 ± 1.2	855
NADPH	21 ± 2.5 µM		72 ± 2.3	3428
α-ketoglutarate	4.6 ± 0.6 µM		61.5 ± 1.7	13369
$GltAB_{complex}$				
Glutamine	65.2 ± 7.7 µM		80.6 ± 2.3	1236
NADPH	10 ± 1.7 µM		90 ± 1.9	9000
α-ketoglutarate	3.5 ± 0.4 µM		80.6 ± 1.8	23028

Supplementary Data Table 3. List of metabolites identified using LC-MS analysis from the GudB-GltAB complex directly isolated from *B. subtilis*.

Metabolite	**Mass (in Da)**	**Retention time (min)**	**Intensity**
Aspartate	132.0302	7.86/8.28	2.5×10^5
Glutamate	146.0459	32/7.89	1×10^5
Unknown	101.0244	8.79	4.5×10^5
Valine	116.0717	5.53	1.84×10^4
α-Ketoglutaric acid	145.0143	8.81	3×10^6

Citrate	191.0197	10.46	1.8×10^5
Succinate	117.0193	8.60	1×10^5
Fumarate	115.0037	9.24	5.5×10^4
Malate	133.0143	9.24	1.7×10^6
Phosphoenolpyruvate	166.9751	10.44	3×10^5
Arginine	173.10	13.72	1.5×10^4

Supplementary Data Table 4. List of potential interactions between GltA and GudB. Noted are the PDB codes and chain identifiers for which the residue number relate. # Residues that are different between RocG and GudB. * Residues on the "minor" GudB subunit that interacts less extensively with GltA. H-bond, hydrogen bond; SB, salt bridge.

RocG (3k92A)	GudB 7MFMA,*7MFMB	GltA (7MFMG)	Bond type	Distance (Å)
R124	R124	K573	H-bond	2.94
N231	N231	N447	H-bond	2.77
D270	D270	K330	SB	3.06
R272	R272	Q445	H-bond	2.41
D273	D273	Y560	H-bond	3.34
S274	S274	K558	H-bond	3.30
N280#	**K280**	K708,E324	H-bond, SB	3.01,3.08
R84	R84	E451	SB	3.45
H86	H86	E454	SB	3.66
K271#	**R271**	E324	SB	3.00
H398#	***R398**	E551, S550	SB,	3.66
K399#	***R399**	Y433	H-bond/Cation-Pi	2.97
R419	*R419	D579	SB	2.56

Supplementary Table 5: Cryo-EM data collection, processing, and model refinement statistics

	$GudB_6$-$GltA_2B_2$ (EMDB-EMD23817) (PDB 7MFM)	$GudB_6$-$GltA_6B_6$ (EMDB-23825) (PDB 7MFT)
Data collection and processing		
Magnification	105,000 X	105,000 X
Voltage (kV)	300 kV	300 kV
Electron exposure (e–/Å^2)	61	47.7
Defocus range (μm)	0.3-2.8	0.3-2.3
Pixel size (Å)	0.824	0.824
Symmetry imposed	C2	D3
Initial particle images (no.)	1353359	60487
Final particle images (no.)	718672	11878
Map resolution (Å)	2.42	3.9
FSC threshold	0.143	0.143
Map resolution range (Å)	2.4-3.6	3.0-7.5
Refinement		
Initial model used (PDB code)	1OFD, 6S6T, 3K8Z	GudB1-GltA1B1 (7MFM)
Model resolution (Å)	2.5	3.9
FSC threshold	0.143	0.143
Model resolution range (Å)	2.4-3.6	3.0-7.5
Map sharpening *B* factor (Å^2)	0.0	0.0
Model composition		
Non-hydrogen atoms	50242	18733
Protein residues	6406	2383
Ligands	10	79.80
B factors (Å^2)		
Protein	94.00	97.77
Ligand	106.93	79.80
R.m.s. deviations		
Bond lengths (Å)	0.003 (0)	0.003 (0)
Bond angles (°)	0.534	0.559
Validation		
MolProbity score	1.88	1.76
Clashscore	5.17	6.82
Poor rotamers (%)	2.10	0.00
Ramachandran plot		
Favored (%)	94.79	94.36
Allowed (%)	5.12	5.60
Disallowed (%)	0.09	0.04

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57 **Supplementary Table 6. *B. subtilis* strains used in the study**

Strain name	Genotype	Description
3610	Prototroph	Wild-type *Bacillus subtilis*
gltB-TS	3610 *gltB*::*gltB-TS*	GltB, under its native promoter, tagged with a twinstrep (TS)-at its C-terminus. Used for pulldown of GltB and of the GudB-GltAB complex for kinetic studies.
gltB-TS-P_{gudB}-*rocG*	3610 *gltB*::*gltB-TS rocG::kan amyE ::* (*P_{gudB}-rocG-spec*)	As above, *plus* RocG placed under GudB's promoter (at the *amyE* landing site). Endogenous rocG is knocked out with a kanamycin cassette. Used for testing the in vivo interaction capability of RocG with GltAB
Δ*gudB*	3610 *gudB*::*erm*	Knockout of *gudB*. Used for growth profiling in media containing different C/N source
Δ*gltA*	3610 *gltA*::*erm*	Knockout of *gltA*. Used for growth profiling in media containing different C/N source
Δ*gltB*	3610 *gltB*::*erm*	Knockout of *gltB*. Used for growth profiling in media containing different C/N source
Δ*gltABC*	3610 *gltABC*::*erm*	A knockout of the entire *gltABC* operon. Used for the generation of all the following strains.
P_{hs}-*gltAB*^{C1A}	3610 *gltABC* :: (*P_{hs}-gltA^{C1A}-gltB-TS-cat*)	Strain expressing the inactive mutant of GltA (GltA^{C1A}) along with a GltB-TS from an IPTG inducible hyper spank promoter (P_{hs}). GudB is expressed from its endogenous promoter (P_{gudB}). Used for planktonic and biofilm growth phenotyping and for the purification of the GudB-GltAB^{C1A} complex.

P_{hs}-*gltAB*^{C1A}+P_{gudB}-*rocG*	3610 *gltABC* :: (*P_{hs}-gltA*^{C1A}*-gltB-TS-cat*) *rocG*::*kan amyE* :: (*P_{gudB}rocG-spec*)	Strain expressing the inactive mutant of GltA (GltA^{C1A}) along with a GltB-TS from P_{hs}. The strain also constitutively expresses RocG using P_{gudB} from *amyE* locus. Used for planktonic and biofilm growth phenotyping and for the purification of the GudB-GltAB^{C1A} complex. The strain was also used to test the *in vivo* interaction capability of RocG with GltAB^{C1A}.
P_{hs}-*gltAB*	3610 *gltABC* :: (*P_{hs}-gltA-gltB-TS*)	Strain expressing GltA along with a GltB-TS from P_{hs}. Used for overexpression and purification of functional GudB-GltAB complex for structural studies. The strain was also used for planktonic and biofilm growth phenotyping.
P_{hs}-*gltAB*+P_{gudB}-*rocG*	3610 *gltABC* :: (*P_{hs}-gltA-gltB-TS*) *rocG*::*kan amyE* :: (*P_{gudB}rocG-spec*)	Strain expressing GltA along with a GltB-TS from P_{hs}. The strain also constitutively expresses RocG using P_{gudB} from the *amyE* locus. The strain was also used for planktonic and biofilm growth phenotyping.
P_{hs}-*gltAB* - Δ*gudB*	3610 *gltABC* :: (*P_{hs}-gltA-gltB-TS*) *gudB*::*erm*	Strain expressing GltA along with GltB-TS from P_{hs} but devoid of GudB. For overexpression and purification of GltAB without co-purification of GudB.

Supplementary Table 7. Oligonucleotides used in the study

Each row of the table provides the name of the construct, the fragments used to generate the construct and oligonucleotide sequence used to generate the respective fragment. 5HR and 3HR corresponds to the 5' and 3' homology arms for the recombination.

Fragments	**Sequence (5'-3')**
gltB-TS	
a.*colE1-ampR*	**5'** atccggtaaagtgcactggagttctttagcaaatttttttattag **3'**
	5' agaatctcaggtacatcttgacactccttatttgatttttttgaag **3'**
b.5HR	**5'** cttcaaaaaatcaaataaggagtgtcaagaaatgtctgaaacgcactcgtaataaaaaagtctttcc**3'**
c.*gltB-TS*	**5'** tgcctggtctcatccacaatttgagaataaataaaggggattatcatataagagaaaccggtctggctgc**3'**
	5'gataatccccttttatttattctcaaattgtggatgagaccaggcagaacctcccgatccacctccggaacctccacctttttcgaattgtggatgactccaggaacccggaagaactgaactccccatcaaatatcg**3'**
d. 3HR	**5'** gtagtacagtaaggaaggggagaggaacatggggaaaccaactggatttatggagatcaaacg**3'**

	5' ctccataaatccagttggtttccccatgttcctctccccttccttactgtactaccgc**3'** **5'**ctaataaaaaaatttgctaaagaactccagtactgagggcgtcgtaaaagtgatgtccaagatgggaatttcaactg tgc**3'**
Δ*gltA*	
a.*colE1-ampR*	**5'** tattaattgatggggttgctggagttctttagcaaatttttttattagctgaacttag**3'** **5'** atgggccggttgaacacgtcttgacactccttatttgatttttgaagacttacttcg**3'**
b.5HR	**5'** aataaggagtgtcaagacgtgttcaaccggcccatatacaaaagcgccatcaacttt**3'** **5'** tgccagagttaaaggatcggaaggggagaggaacatggggaaaccaactggatttatg**3'**
c.Erm	**5'** tgttcctctccccttccgatcctttaactctggcaaccctcaaaattgaatgagacatg**3'** **5'** atcgggggagaggaatttggtttaagccgactgcgcaaaagacataatcgattcaca**3'**
d. 3HR	**5'** cgcagtcggcttaaaccaaattcctctcccccgatcaatttccgataataccggtcataaaatc**3'** **5'** ttgctaaagaactccagcaaccccatcaattaatattgtcggtccttgccggcataa**3'**
ΔgltABC	
a.5HR	**5'** cgaacgcaaagccggagacatcattaatat**3'** **5'** cagagttaaaggatcaaaaaatgaacccgagcttctatatagaagc**3'**
b.Erm	**5'** tcgggttcatttttgatcctttaactctggcaaccctcaaaattg**3'**
c.3HR	**5'** gataatccccttattggtttaagccgactgcgcaaaagacataa**3'** **5'** agtcggcttaaaccaataaaggggattatcatataagagaaaccg**3'** **5'** tgcaaatgctgaaaatccttgatcattttaac**3'**
Δ*gltB*	
a.*colE1-ampR*	**5'** atccggtaaagtgcactggagttctttagcaaatttttttattag**3'** **5'** agaatctcaggtacatcttgacactccttatttgatttttgaag**3'**
b.5HR	**5'** taaggagtgtcaagatgtacctgagattctgggaggtccttcgga**3′**
c.*erm*	**5'** cagagttaaaggatcataaaggggattatcatataagagaaaccggtctggc**3′**
d.3HR	**5'** gataatccccttttatgatcctttaactctggcaaccctcaaaattg**3'** **5'** gaaggggagaggaactggtttaagccgactgcgcaaaagacataa**3'**
	5' agtcggcttaaaccagttcctctccccttccttactgtactaccgc**3'** **5'** gctaaagaactccagtgcactttaccggatcagccggccaaagct**3'**
Δ*gudB*	
a.*ampR*	**5'** catcaggctcgtactggagttctttagcaaatttttttattag**3'** **5'** aaaatcggaacaatcttgacactccttatttgatttttgaag**3'**
b.5HR	**5'** ggagtgtcaagattgttccgattttatcatgaagctgatccac**3'**
c.*erm*	**5'** gagttaaaggatcgttgatgatttgcataaaaataaaaaatctcctatgataaaatag**3'**
d.3HR	**5'** caaatcatcaacgatcctttaactctggcaaccctcaaaattg**3'** **5'** aggttaactcaatggtttaagccgactgcgcaaaagacataa**3'**
	5' tcggcttaaaccattgagttaacctcctagaatcttctgtttctcacatgctccc**3'**

	5' aaagaactccagtacgagcctgatgacggaaacctcccgtgc**3'**
P_{hs}-gltAB	
a.5HR	**5'** aataaggagtgtcaagacgaacgcaaagccggagacatcattaatatttcatctacagcgggccaaa**3'** **5'** gagtgctcacatttaccctgaaagtacttttgctgaaacaacacctcgttttactgtgaaaattccaattgag**3'**
b.P_{hs}	**5'** cagcaaaagtactttcagggtaaatgtgagcactcacaattcattttgcaaaagttgttgactttat**3'** **5'** atttgattgtacgtcatgtttgtcctccttattagttaatcagctagctgtcgactaagcttaattg**3'**
c.*gltAB-TS*	**5'** ctaataaggaggacaaacatgacgtacaatcaaatgccaaaagctcaaggtctctaccgtcctgaatt**3'** **5'** attagcttgcatgcaaaaccggtttctcttatatgataatccccttttatttattctcaaattgtggatgagac**3'**
d.*lacI*	**5'** catataagagaaaccggttttgcatgcaagctaattcggtggaaacgaggtcatcatttccttccgaaaaaac**3'** **5'** tatgataatcccctttattggatttccttacgcgaaatacgggcagacatggcctgcccggttattat**3'**
e.3HR	**5'** tcgcgtaaggaaatccaataaaggggattatcatataagagaaaccggtctggctgccagccggttt**3'** **5'** ttgctaaagaactccagtgcaaatgctgaaaatccttgatcattttaacctttcacaatacgatgtccg**3'**
f. *colE1*	**5'** ggattttcagcatttgcactggagttctttagcaaatttttttattagctgaacttagtattagtggc**3'** **5'** tctccggctttgcgttcgtcttgacactccttatttgatttttgaagacttacttcggagtcaaaaa**3'**
P_{hs}-*gltAB*^{C1A}	
a. 5HR	**5'** tttcgtcttcaagaacgaacgcaaagccggagacatcattaatatttcatctaca**3'** **5'** tgctcacatttaccctgaaagtacttttgctgaaacaacacctcgttttactgtg**3'**
b. P_{hs}-*gltAB-TS*	**5'** gcaaaagtactttcagggtaaatgtgagcactcacaattcattttgcaaaagttg**3'**
c. cat	**5'** cattatgtactatttttattctcaaattgtggatgagaccaggcagaacctcccg**3'**
d. lacI	**5'** acaatttgagaataaaaatagtacataatggatttccttacgcgaaatacgggcagacatgg**3'** **5'** tagcttgcatgcaaactcccttatgcgactcctgcattaggaagcagcccagtag**3'**
e. 3HR-*colE1*	**5'** agtcgcataagggagtttgcatgcaagctaattcggtggaaacgaggtcatcatttcc**3'** **5'** gataatcccctttattggatttccttacgcgaaatacgggcagacatggcctgcc**3'**
	5' gcgtaaggaaatccaataaaggggattatcatataagagaaaccggtctggctgc**3'** **5'** ccggctttgcgttcgttcttgaagacgaaagggcctcgtgatacgcctatttttta**3'**