

## SUPPORTING INFORMATION

### **Functional dissection of N-acetylglutamate synthase (ArgA) of *Pseudomonas aeruginosa* and restoration of its ancestral N-acetylglutamate kinase activity**

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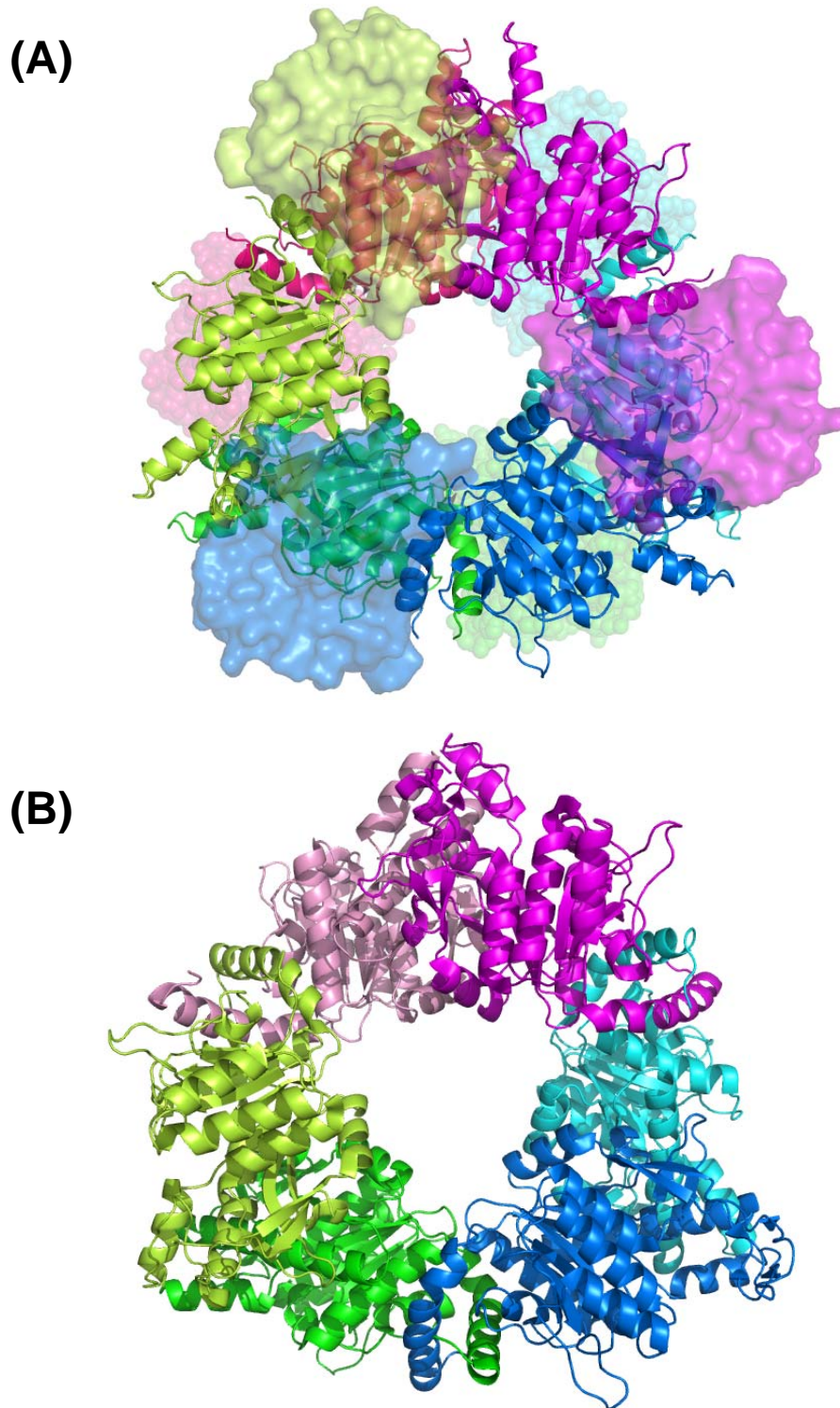
Supplementary Figure S1

Supplementary Table S1

**Supplementary Table S1.** Synthetic oligonucleotides used in this work. Mutations are underlined. The ATG for the initial Met is in bold-type. Restriction sites are highlighted in grey background. Italic lettering marks the beginning or the end of the open reading frame.

	Direction	Sequence
<b><i>Whole domains</i></b>		
GNAT	Forward	5'GCCCAGGAGCAGCAT <b>ATG</b> CAGTTGCGCGAGGCG 3'
	Reverse	5'GGACGCCTCGAGGGATCCAGGCTCTTCTCG 3'
rGNAT	Forward	5'GTTTAACTTTAAGAAGGGTCTCTAGGT <b>ATG</b> CAGTTGCGCGAGGCG3'
	Reverse	5'GTGGTGCTCGAGGGATCCTTACAGGCTCTTCTCGAATACC 3'
rAAK	Forward	5'GCAGTTCGAGTAGTTGCGCGAGGCGGGC 3'
	Reverse	5'CGCCTCGCGCAACTACTCGAACTGCTC 3'
$\Delta$ 1-12 <sup>a</sup>	Forward	5'GGTTACGTCACC <b>ATATG</b> CCCTACATCAACTCG 3'
<b><i>Linker changes<sup>b</sup></i></b>		
ERM	Forward	5'GGTCGCCCAGGAGCGGATGGAGCAGTTGCG 3'
	Reverse	5'CAACTGCTCCAICCGCTCCTGGGCGACCAG 3'
AQF	Forward	5'CTGGTCGCCCAGGCACAGTTCGAGCAGTTG 3'
	Reverse	5'CTGCTCGAACTGTGCCTGGGCGACCAGGG 3'
EQM	Forward	5'GCCCAGGAGCAGATGGAGCAGTTGCGCGAG 3'
	Reverse	5'GCGCAACTGCTCCAICTGCTCCTGGGCGAC 3'
ERF	Forward	5'GCCCAGGAGAGGTTTCGAGCAGTTGCGC 3'
	Reverse	5'CAACTGCTCGAACCTCTCCTGGGCGAC 3'
EAQGP	Forward	5'CAGGAGGCACAGGGTCCCGAGCAGTTGCGCGAG 3'
	Reverse	5'GCGCAACTGCTCGGGACCCTGTGCCTCCTGGGC 3'
LFQGP	Forward	5'CTGGTCGCCCAGCTGTTTCAGGGTCCCGAGCAGTTG 3'
	Reverse	5'CTGCTCGGGACCCTGAAACAGCTGGGCGACCAGGG 3'
LEVLQGP	Forward	5'GCACCCTGGTCGCCCAGCTGGAAGTTCTGTTTCAGGGTCCCGAGC3'
	Reverse	5'CGGGACCCTGAAACAGAACTTCCAGCTGGGCGACCAGGGTGCCG3'
<b><i>rAAK point mutations</i></b>		
L240K	Forward	5'GCTACCAGGCCGAGAAAGCTGGACGCCGCG 3'
	Reverse	5'GGCGTCCAGCTTCTCGGCCTGGTAGCTG 3'
M26K	Forward	5'CACTTCGTGGTCAAGCTCCCCGGCGAAGGG 3'
	Reverse	5'CTTCGCCGGGGAGCTTGACCACGAAGGTGCG 3'

<sup>a</sup>This forward oligonucleotide was combined with the GNAT domain reverse oligonucleotide. <sup>b</sup>Changes as defined in Fig. 2B.



**Supplementary Figure S1.** The structures of (A) the NAGS from *N. gonorrhoeae* (PDB file 2R8V) and (B) of NAGK from *P. aeruginosa* (PDB file 2BUF) to illustrate grossly their structural similarity. They are viewed along their threefold axes, with each dimer colored differently and both subunits of each dimer in different color hues. AAK and GNAT domains are shown, respectively, in cartoon and surface representation. In the case of NAGS, to avoid occluding the view of the AAK domains, the GNAT domains are semi-transparent, and those in the background are fainter.