



Figure S1 Predicted structure of *P. aeruginosa* PA0320 (CarO) (A and B) and PA0327 (CarP) (C and D). Structural predictions were made by using i-TASSER (8) and HHpred (9), and visualized by using PyMOL (The PyMOL Molecular Graphics System, Version 1.7.6 Schrödinger, LLC). The structural predictions for CarO is based on threading onto putative lipoprotein of *Clostridium difficile* 630 (PDB#4EXR). CarO is predicted to form a OB fold domain as highlighted in magenta (A and B). The structure is rotated 90° around the y-axis (B). The structural predictions for CarP were based on threading onto the *E. coli* YjiK (PDB#3QQZ). CarP is predicted to form a  $\beta$ -propeller, with the  $\beta$ -sheets highlighted in blue. The amino acid residues predicted to bind  $\text{Ca}^{2+}$  are within the central pocket, and are highlighted in red. (A) Top view of the predicted structure. (B) 90° rotation around the x-axis showing the side view of the  $\beta$ -propeller structure. Rotating the structure reveals that the predicted  $\text{Ca}^{2+}$  binding residues are located at one entrance of the  $\beta$ -propeller tunnel.

**Table S1. Bacterial Strains, plasmids and Oligonucleotide Sequences**

Strains/Plasmids	Description	Reference
<i>P. aeruginosa</i>		
PAO1	Wild type strain, Alg <sup>-</sup>	(1)
FRD1	Cystic Fibrosis isolate, Alg <sup>+</sup>	(2)
PA0320-H07::ISlacZ/hah	PAO1 with Tn5 disruption of PA0320	(3)
PA0327-B11::ISphoA/hah	PAO1 Tn5 disruption of PA0327	(3)
$\Delta carR::Gm$	PAO1 with deletion of <i>carR</i> replaced with Gm <sup>r</sup> gene	This study
PA0320-H07::ISlacZ/hah/P <sub>BAD</sub> -PA0320	Tn5 disruption of PA0320 complemented by P <sub>BAD</sub> -PA0320	This study
PA0327-B11::ISphoA/hah/ P <sub>BAD</sub> -PA0327	Tn5 disruption of PA0320 complemented by P <sub>BAD</sub> -PA0327	This study
$\Delta carR::Gm/ P_{BAD-carRS}$	Deletion of <i>carR</i> complemented by <i>cars</i>	This study
Plasmids		
pTJ1	Tn7 vector containing P <sub>BAD</sub> promoter, Tm <sup>r</sup>	(4)
pMMB66EH-AEQ	pMMB66EH containing aequorin gene from <i>Aequorea Victoria</i>	(5)
pTNS1	Helper plasmid for Tn7 transposase, Ap <sup>r</sup>	(6)
pFLP2	Helper plasmid for FLP recombinase, Ap <sup>r</sup>	(7)
pCR2.1	Cloning vector from Invitrogen, Ap <sup>r</sup> , Km <sup>r</sup>	Invitrogen
pTA53	PA0320 in pCR2.1	This study
pTA54	PA0327 in PCR2.1	This study
pTA55	P <sub>BAD</sub> -PA0320 in pTJ1	This study
pTA56	P <sub>BAD</sub> -PA0327 in pTJ1	This study
pTA57	PA2656 in pCR2.1	This study
pTA99	PA2657 in pCR2.1	This study
pTA104	P <sub>BAD</sub> -PA2656 and 2657 in pTJ1	This study
Oligonucleotides		

aequorin – For	CTTACATCAGACTTCGACAACCCAAG
aequorin – Rev	CGTAGAGCTTCTTAGGGCACAG
PA0320 NcoI 5'	<u>ACCCATGGAACTTCGTCACCT</u>
PA0320 XbaI 3'	GGTCTAGAAGGGGTTCAACCGG
PA0327 NcoI 5'	TGCCATGGCTATCCACGCCCA
PA0327 XbaI 3'	AGTCTAGAGCGGGCCAGGGG
5'PA2657-EcoRI	GCAGAATTCATCCTGAAGGACGAGGAAGACGAC
3'PA2657	GTCCAGTTGCATGCCGTTTCG
5'PA2656	CCAGTGCGTGTGCCTGAACG
3'PA2656-EcoRV	CGAGATATCCGGGACTGTCCGGCAAATGGT

RT-qPCR Primers

0320Left_RtPCR	GACACCCCGGTGGTCTCCA
0320Right_RtPCR	TCGACTTCGCCGGTCAGCTTG
0327Left_RtPCR	CCGGTCGGGATCGAAGGTCA
0327Right_RtPCR	GGTTCGCCGTCCAGGAGTGG
acpLeftRT-PCR2	ACTCGGCGTGAAGGAAGAAG
acpRightRT-PCR2	CGACGGTGTCAAGGGAGT

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