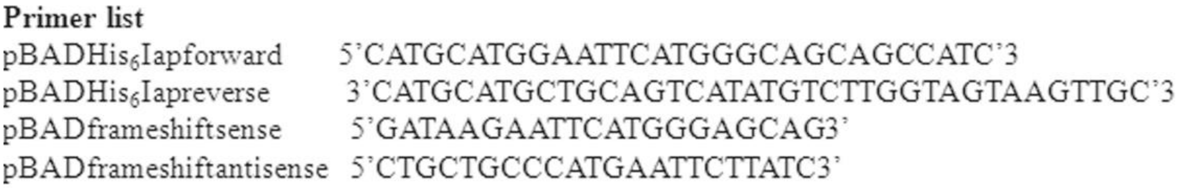
The D3 bacteriophage α-polymerase-inhibitor (Iap) peptide disrupts O-antigen biosynthesis through mimicry of the chain length regulator Wzz in *Pseudomonas aeruginosa*.

Véronique L. Taylor, Molly L. Udaskin, Salim T. Islam and Joseph S. Lam

Supplementary Information



Primer list

pBADHis$_6$Iapforward	5'CATGCATGGAATTCATGGGCAGCAGCCATC'3
pBADHis$_6$Iapreverse	3'CATGCATGCTGCAGTCATATGTCTTGGTAGTAAGTTGC'3
pBADframeshiftsense	5'GATAAGAATTCATGGGAGCAG3'
pBADframeshiftantisense	5'CTGCTGCCCATGAATTCTTATC3'

FIG. S1. List of primers used in this study.

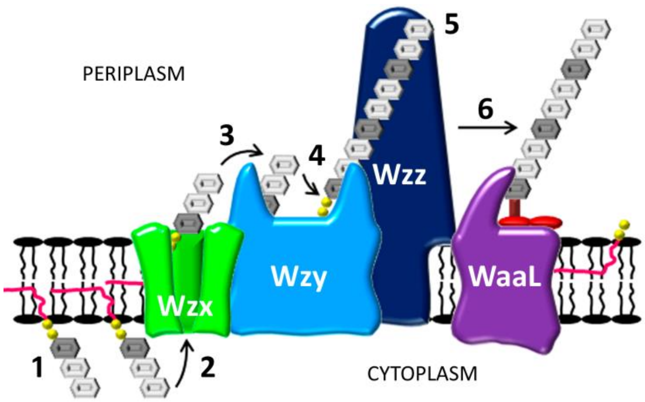


FIG. S2. Schematic representation of the Wzx/Wzy-dependent biosynthesis pathway in *P. aeruginosa* PAO1.

1. Polysaccharide repeat precursors are synthesized on the cytoplasmic face of the IM to the lipid carrier undecaprenyl pyrophosphate.

2. The complete trisaccharide repeat unit is flipped by action of Wzx to the periplasmic side of the IM.

3. Wzy-mediated polymerization of the OSA chain by the addition of newly synthesized repeats to the reducing terminus of the growing chain.

4. The growing polysaccharide chain is regulated by Wzz which imparts specific modal lengths.

5. The completed chain is ligated to the lipid-A core oligosaccharide to form a mature LPS molecule, ready to be exported to the OM.

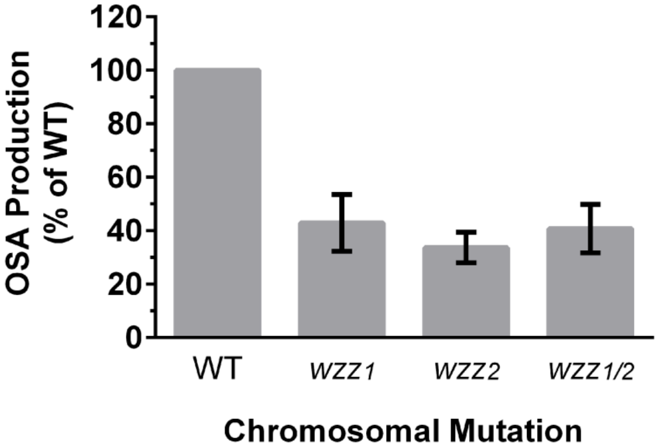
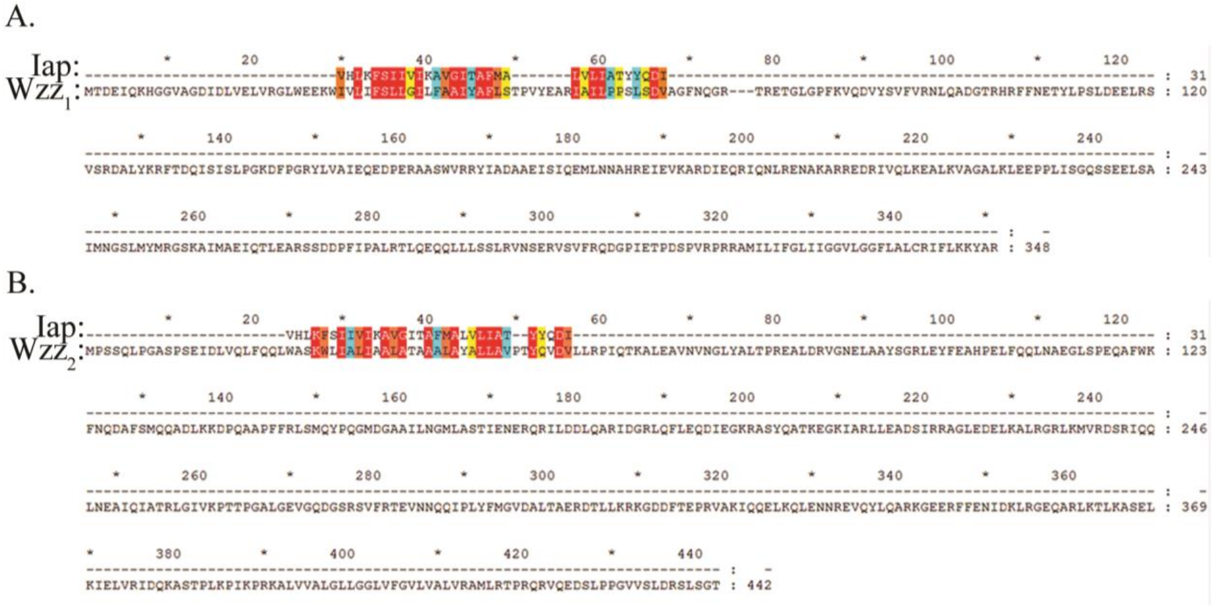


FIG. S3. Densitometry analysis of the OSA levels from *P. aeruginosa* PAO1 and each *wzz* chromosomal mutant strain. The ratio of OSA density to that of inner oligosaccharide from six biological replicates ($n = 6$) was compared and plotted as a percent of WT. Differences in values for each of the *wzz* mutants compared to the WT were shown to be statistically significantly via the Student's t-test (WT—wzz_1, p =0.0003; WT—wzz_2, p = <0.0001; WT—wzz_1-wzz_2, p = <0.0001). Comparison between *wzz* mutant strains indicated that any differences were not statistically significant (wzz_1—wzz_2, p = 0.4615; wzz_1—wzz_1-wzz_2, p = 0.8795; wzz_2—wzz_1-wzz_2, p = 0.5237).



A.

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Iap:  -----------------------------VHLKFSIIVIKAVGITAFMA-------IVLIATYYQDI-------------------------------------------------------- :  31
Wzz1: MTDEIQKHGGVAGDIDLVELVRGLWEEKWIVLIFSLIGILFAAIYAFLSTPVYEARIAIIPPSLSDVAGFNQGR---TRETGLGPFKVQDVYSVFVRNLQADGTRHRFFNETYLPSLDEELRS : 120

      --------------------------------------------------------------------------------------------------------------------------- :   -
      VSRDALYKRFTDQISISLPGKDFPGRYLVAIEQEDPERAASWVRRYIADAAEISIQEMLNNAHREIEVKARDIEQRIQNLRENAKARREDRIVQLKEALKVAGALKLEEPPLISGQSSEELSA : 243

      --------------------------------------------------------------------------------------------------------- :   -
      IMNGSLMYMRGSKAIMAEIQTLEARSSDDPFIPALRTLQEQQLLLSSLRVNSERVSVFRQDGPIETPDSPVRPRRAMILIFGLIIGGVLGGFLALCRIFLKKYAR : 348
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B.

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Iap:  -----------------------VHLKFSIIVIKAVGITAFMALVLIAT--YYQDI------------------------------------------------------------------- :  31
Wzz2: MPSSQLPGASPSEIDLVQLFQQLWASKWLIALIAALATAAALAYALLAVPTYQVDVLLRPIQTKALEAVNVNGLYALTPREALDRVGNELAAYSGRLEYFEAHPELFQQLNAEGLSPEQAFWK : 123

      --------------------------------------------------------------------------------------------------------------------------- :   -
      FNQDAFSMQQADLKKDPQAAPFFRLSMQYPQGMDGAAILNGMLASTIENERQRILDDLQARIDGRLQFLEQDIEGKRASYQATKEGKIARLLEADSIRRAGLEDELKALRGRLKMVRDSRIQQ : 246

      --------------------------------------------------------------------------------------------------------------------------- :   -
      LNEAIQIATRLGIVKPTTPGALGEVGQDGSRSVFRTEVNNQQIPLYFMGVDALTAERDTLLKRKGDDFTEPRVAKIQQELKQLENNREVQYLQARKGEERFFENIDKLRGEQARLKTLKASEL : 369

      ------------------------------------------------------------------------- :   -
      KIELVRIDQKASTPLKPIKPRKALVVALGLLGGLVFGVLVALVRAMLRTPRQRVQEDSLPPGVVSLDRSLSGT : 442
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FIG. S4. Sequence alignment of Iap to Wzz$_1$ and Wzz$_2$ proteins from *P. aeruginosa* PAO1. No region other than the N-terminal TMS of the Wzz proteins demonstrates any similarity to Iap.

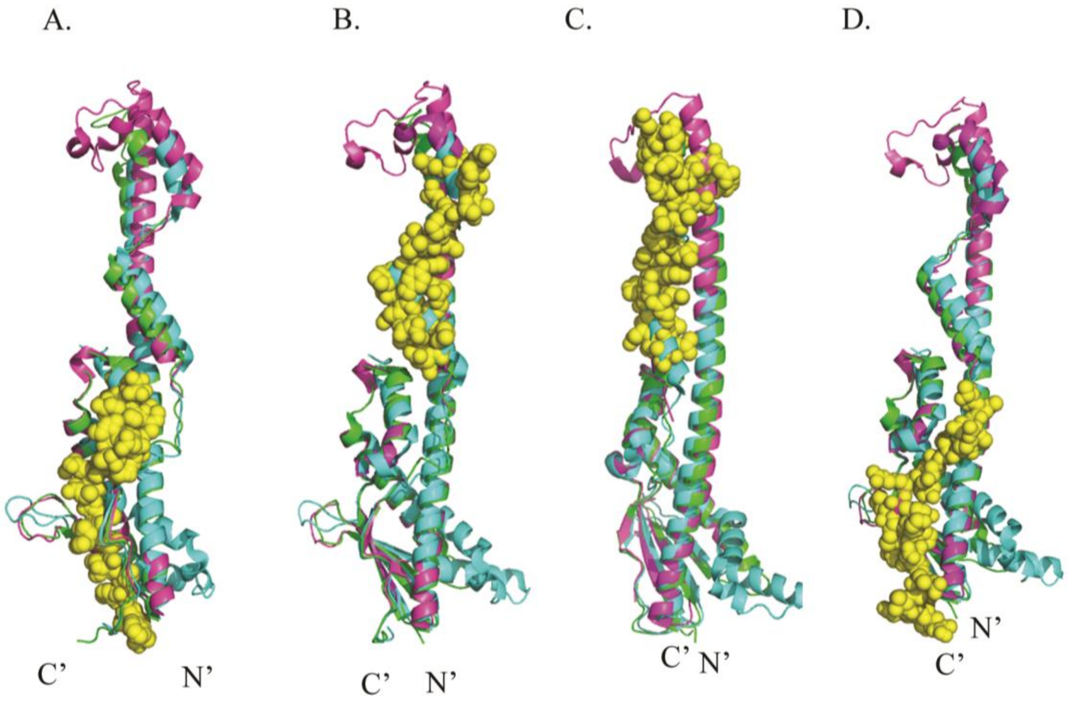

FIG. S5. Localization of Iap alignment to heterologous full-length Wzz proteins. The displayed backbone periplasmic domain of Wzz has been generated by an alignment of the previously solved crystal structures, green: *Salmonella tyhpimurium* (PDB: 3B8P) , fuschia: *E. coli* O157:H7 (PDB: 3B8O) and cyan: *Shigella flexneri* (PDB: 42EH). The yellow spheres designate the region of Iap sequence alignment against Wzz sequence of (A) *V. cholerae* (BAA33593), **(**B) *Salmonella typhimurium* LT2 (AAL20983), **(**C) *E. coli* K-12(AAC75088), and **(**D**)** *P. aeruginosa* PA7 (ABR86688.1). The N and the C termini of the periplasmic domains are labeled.

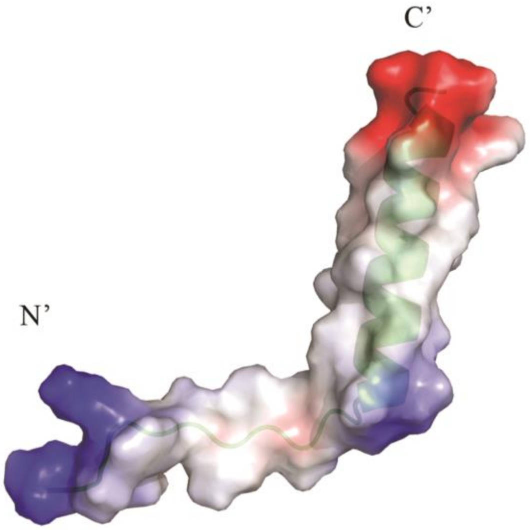

FIG. S6. Surface electrostatics of the Iap tertiary structure model. Color key: *blue*, positive charge; *red*, negative charge; *white*, uncharged/hydrophobic. The N and the C termini are labelled.