Supplementary Materials

The *Pseudomonas aeruginosa* PrrF1 and PrrF2 small regulatory RNAs (sRNAs) promote 2-alkyl-4-quinolone production through redundant regulation of the *antR* mRNA

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Table S1. Strains and plasmids used in this study

Name	Description	Reference
Plasmids		
Mini-CTX1-*lacZ*	Integration-proficient plasmid containing a promoterless *lacZY* gene	(1)
Mini-CTX1-P_{antR}'-*lacZ*	Mini-CTX1-*lacZ* with the *antR* promoter (67 nt upstream of the transcriptional start site) cloned into the MCS	This study
Mini-CTX1-'*lacZ*$^{-SD}$	Mini-CTX1-*lacZ* with the Shine-Dalgarno site deleted	This study
Mini-CTX1-P_{antR}-'*lacZ*$^{-SD}$	Mini-CTX1-*lacZ*$^{-SD}$ with the *antR* promoter (67 nt upstream of the +1 transcriptional start site) and UTR (15 nt downstream of the +1 translational start site) cloned into the MCS	This study
Mini-CTX1-alt-P_{antR}A-'*lacZ*$^{-SD}$	Mini-CTX1-P_{antR}-'*lacZ*$^{-SD}$ with mutations as described in Figure 2 for Alt AntRA	This study
Mini-CTX1-alt-P_{antR}B-'*lacZ*$^{-SD}$	Mini-CTX1-P_{antR}-'*lacZ*$^{-SD}$ with mutations as described in Figure 2 for Alt AntRB	This study
Mini-CTX1-alt-P_{antR}C-'*lacZ*$^{-SD}$	Mini-CTX1-P_{antR}-'*lacZ*$^{-SD}$ with mutations as described in Figure 2 for Alt AntRC	This study
Mini-CTX1-P_{lac}-'*lacZ*$^{-SD}$	Mini-CTX1-'*lacZ*$^{-SD}$ with the native P_{lac} promoter inserted into the MCS	Susana Maurino-Lopez and Angela Wilks
Mini-CTX1-P_{lac}-UTR_{antR}-'*lacZ*$^{-SD}$	Mini-CTX1-P_{lac}-'*lacZ*$^{-SD}$ with the *antR* UTR (from the +1 transcriptional start site to 15 nt downstream of the +1 translational start site) cloned into the MCS	This study
Strains		
Top 10 *E. coli*	F-*mcrA* Δ(*mrr*-hsdRMS-*mcr*BC) Φ80*lac*ZΔM15 ΔlacX74 recA1 *a*raD139Δ(*araleu*)7697 *galU galK rpsL* (StrR) *endA1 nup*G	Invitrogen
DH5 α	*F– Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rK–, mK+) phoA supE44 λ– thi-1 gyrA96 relA1*	(2)
SM10 λ*pir*	*E. coli* strain used for conjugation: pirR6K	(2)
SM10/pFLP	SM10 carrying the pFLP recombinase	(3)
PAO1	*P. aeruginosa* laboratory strain	(4)
Δ*prrF1*	*prrF1* deletion in PAO1	(5)
Δ*prrF2*	*prrF2* deletion in PAO1	(5)
Δ*prrF1,2*	*prrF1,2* deletion in PAO1	(5)
PAO1/P_{antR}-'*lacZ*	PAO1 with the P_{antR}-'*lacZ* reporter fusion integrated at the chromosomal *att* site	This study
Δ*prrF1*/P_{antR}-'lacZ^{-SD}	Δ*prrF1* with the P_{antR}-'*lacZ* reporter fusion integrated at the chromosomal *att* site	This study
Δ*prrF2*/P_{antR}-'lacZ^{-SD}	Δ*prrF2* with the P_{antR}-'*lacZ* reporter fusion integrated at the chromosomal *att* site	This study
Δ*prrF1,2*/P_{antR}-'lacZ^{-SD}	Δ*prrF1,2* with the P_{antR}-'*lacZ* reporter fusion integrated at the chromosomal *att* site	This study
PAO1/altP_{antR}A-'*lacZ*	PAO1 with the altered P_{antR}A-'*lacZ* reporter fusion integrated at the chromosomal *att* site	This study
PAO1/altP_{antR}B-'*lacZ*	PAO1 with the altered P_{antR}B-'*lacZ* reporter fusion	This study

Name	Description	Reference
	integrated at the chromosomal *att* site	
PAO1/altP_{antR}C-'*lacZ*	PAO1 with the altered P_{antR}C-'*lacZ* reporter fusion integrated at the chromosomal *att* site	This study
PAO1/P_{antR}-*lacZ*	PAO1 with the P_{antR}-*lacZ* transcriptional fusion integrated at the chromosomal *att* site	This Study
Δ*prrF1,2*/P_{antR}-*lacZ*	Δ*prrF1,2* with the P_{antR}-*lacZ* transcriptional fusion integrated at the chromosomal *att* site	This Study
PAO1/P_{lac}-UTR_{antR}-'*lacZ*	PAO1 with the P_{lac}-UTR_{antR}-'*lacZ* translational fusion integrated at the chromosomal *att* site	This Study
Δ*prrF1,2*/P_{lac}-UTR_{antR}-'*lacZ*	Δ*prrF1,2* with the P_{lac}-UTR_{antR}-'*lacZ*^{SD} translational fusion integrated at the chromosomal *att* site	This Study

Table S2. Parameters for Multiple Reaction Monitoring

Quinolone	Q1 *m/z*	Q2 *m/z*	CE (V)	T Lens (V)
C7-PQS	260.1	175.1	29	99
C9-PQS	288.1	175.1	29	125
HHQ	244.1	159.1	30	110
NHQ	272.1	159.1	32	128
HQNO	260.1	159.1	27	114
NQNO	288.1	159.1	30	105
Naladixic Acid	233.1	187.1	25	79

Table S3. Primers used in this study

Name	Sequence
Translational reporter fusion construction	
$LacZ^{-SD}$.for	aagcttatgaccatgattacggattcactggc
$LacZ^{-SD}$.rev	atcgataatttcaccgccgaaaggcgcggtgcc
$antR_{67}$.for	gaattcgaccggcgcttgcggcggac
$antR_{67}$.rev	cgtgtcgagtgccgaacgatgatgcgcacccatcccg
alt-antRA.for	gccgaaccatgatgcgcacccatcccgtcgc
alt-antRA.rev	gcgacgggatgggtgcgcatcatggttcggc
alt-antRB.for	gcacccccagccttcgggtgtcgagtgccg
alt-antRB.rev	cggcactcgacacccgaaggctgggggtgc
alt-antRC.for	cgcgtgccgaaccatggcaaggacccatcccgtcg
alt-antRC.rev	cgacgggatgggtccttgccatggttcggcactcg
antR-UTR.for	ggatccggggagccggccttgcgccggcatat
antR-UTR.rev	aagcttatgggtcctcatcatggtt
Transcriptional reporter fusion construction	
antR-promoter.for	gaattcgagcggcgcttgcggcggacgcttt
antR-promoter.rev	aagcttcgcatcctccactatccggatag
Primers for transcription	
PrrF1.for	gtgtaatacgactcactataggg-caactggtcgcgagatcagcc
PrrF1.rev	aaaaaaagacccggcaaagtgccgggtcaa
PrrF2.for	gtgtaatacgactcactataggg-caactggtcgcgaggccagca
PrrF2.rev	aaaaaaagacccggcaaagtgccgggtcga
5'UTR antR.for	gtgtaatacgactcactataggg-gagccggccttgcgccggca
5'UTR antR.rev	catggagatcgccacgatcggcgacggg
5' RACE	
antR.rev	cctcgacacggccccgcccgac
antR.nested	acgggatgggtcctcatcat

Figure S1. 5' RACE of the *antR* transcriptional start site (TSS). The TSS of the *antR* mRNA (‡) was determined by 5' RACE of RNA isolate from iron-replete conditions as described in the Materials and Methods. Fragments for translational fusions were cloned from -67 upstream of the transcriptional start site (‡) identified in this study to +15 downstream of the translational start site (+). Numbers above the sequence indicate the distance from the transcriptional start site identified in this study. The region predicted to pair with the PrrF sRNAs is underlined and in black. The AntR binding site previously identified by Kim, et al, is underlined in purple. The transcriptional start site previously identified by Wurtzel, et al, is indicated by a (*), and the putative RpoN binding site upstream of the longer transcript start site is underlined in red. Fragments for *antR in vitro* transcription were generated from nucleotides 1 at the transcriptional start site (‡) to +42 downstream of translational start site.

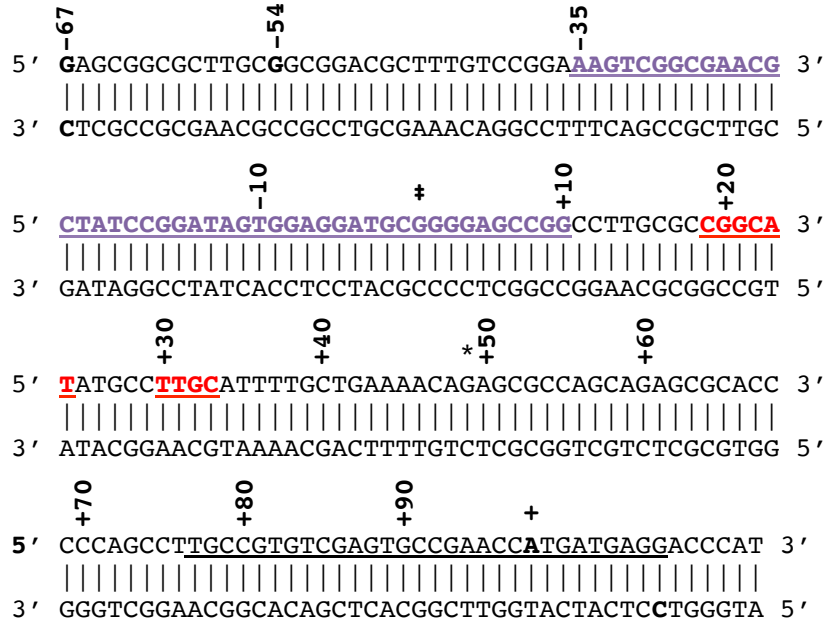


Figure S2. RNase footprinting of PrrF2 sRNA with Pa-Hfq. A-B. Partial digestion of PrrF2 with RNase If in the presence of the indicated amount of *Pa* Hfq protein, as in Figure 4. **B.** SAFA (Semi Automated Footprinting Analysis) analysis of the band intensities. **C.** PrrF2 sRNA secondary structure derived from the RNase partial digestion.

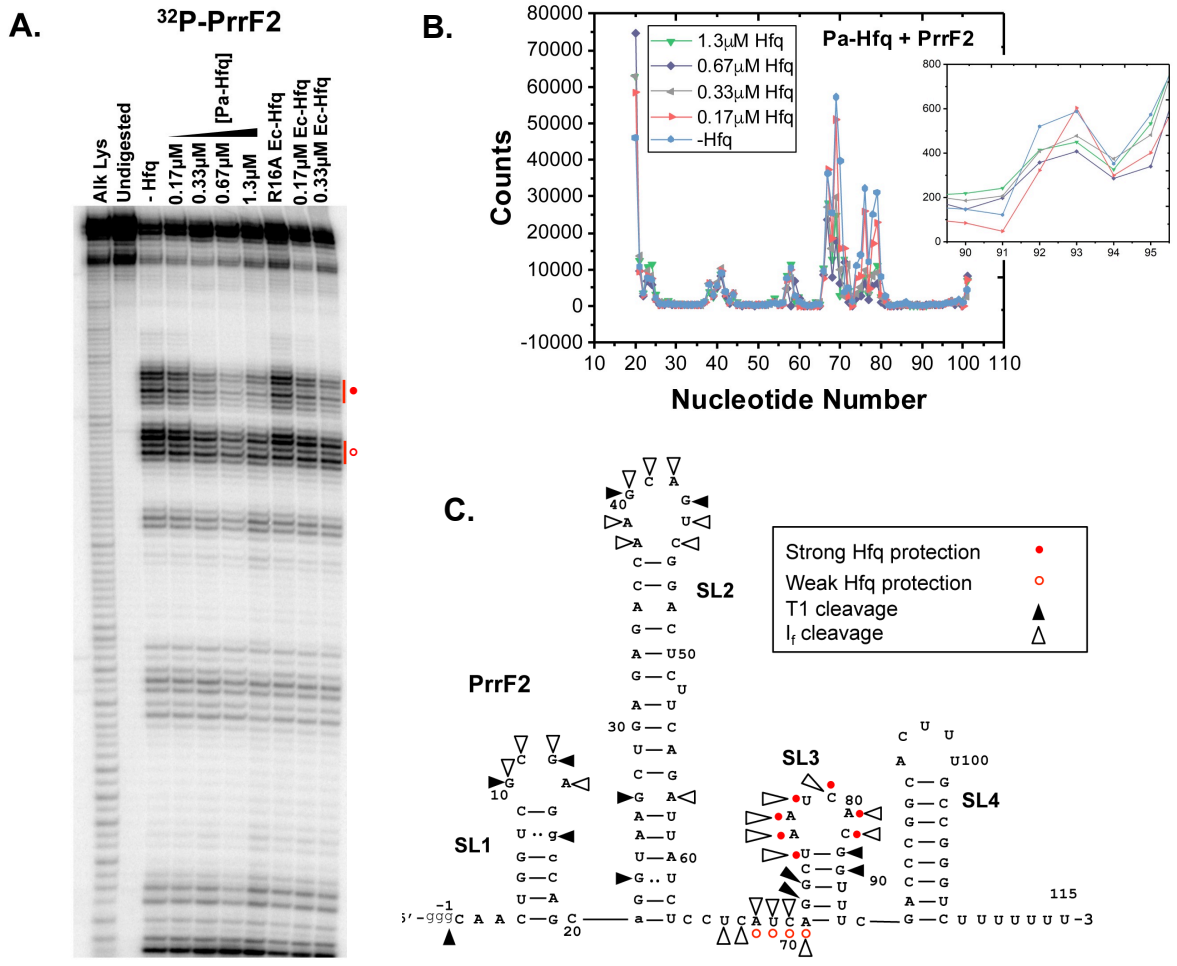


Figure S3. Ec-Hfq protects PrrF1 and PrrF2 at lower concentrations than Pa-Hfq. SAFA (Semi Automated Footprinting Analysis) analysis of RNase protection of PrrF1 (A-C) and PrrF2 (D-F) sRNAs with *Pa* Hfq (A, D), *Ec* Hfq, and R16A *Ec* Hfq proteins (B, E). Regions showing significant protection by the Hfq proteins are expanded in C and F.

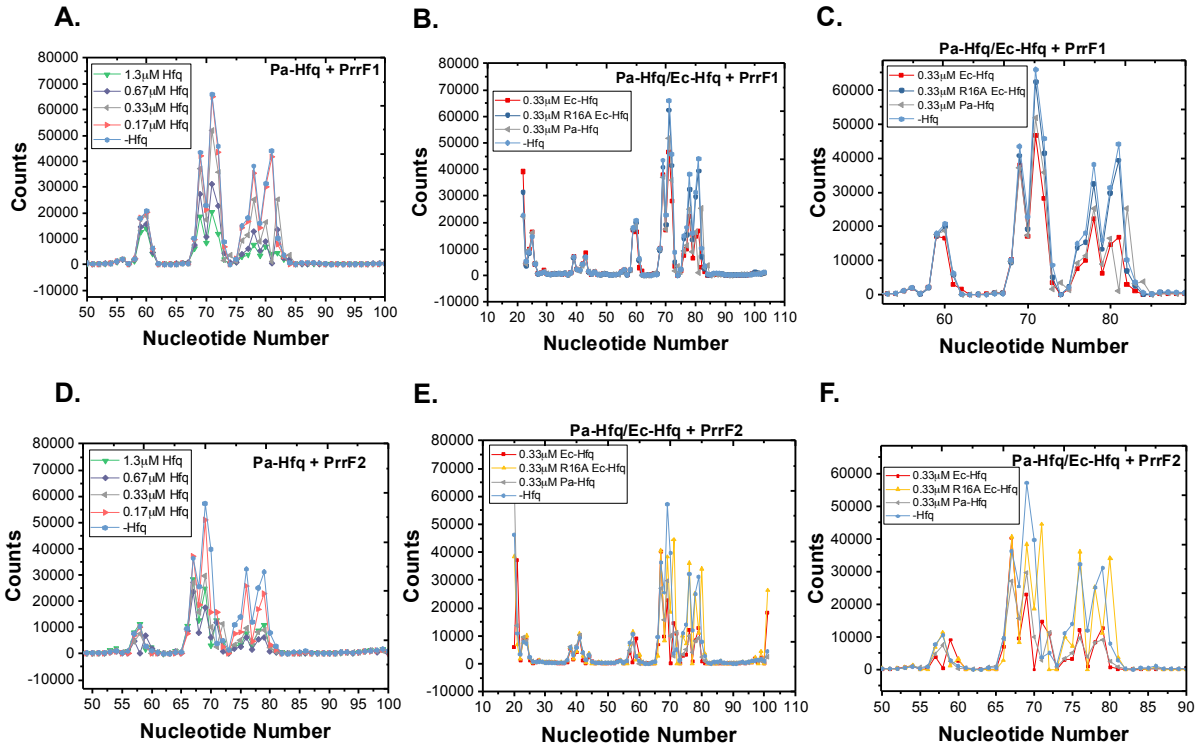


Figure S4. *Pa* Hfq equilibrium binding to PrrF2 sRNA. (A) Incubation of ^{32}P-labeled PrrF2 with 0 – 667 nM Hfq_6. The first shift (P2•H(I)) was due to one hexamer binding to PrrF2 and the second shift (P2•H(II)) was due to two or more hexamers binding to PrrF2. (B) Fractions of P2•H(I) and P2•H(II) complexes were fit to eq. (1) to obtain the dissociation constants.

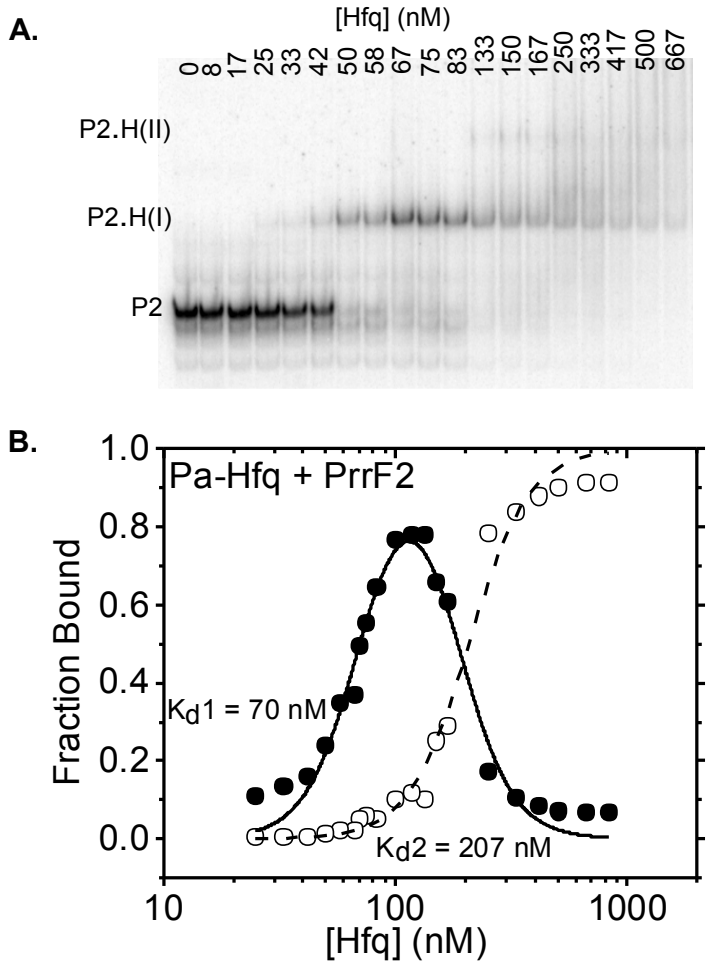
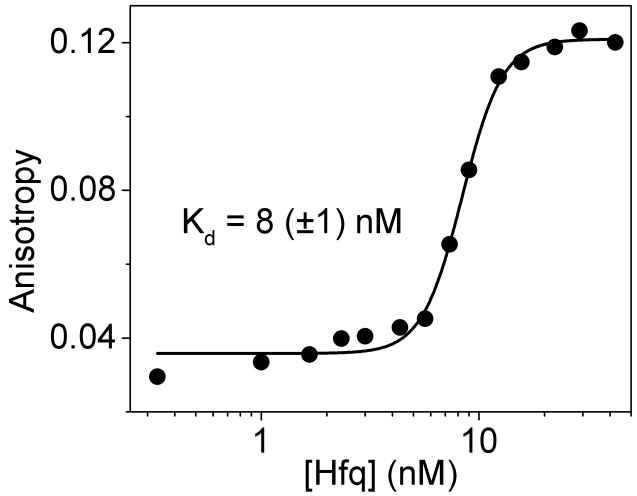


Figure S5. Equilibrium binding of *Pa* Hfq with A18 RNA. The dissociation constant of *Pa* Hfq with an unstructured A18-FAM RNA was determined by fluorescence anisotropy as previously described (6).



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