

## Appendix

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**Appendix Table 1: Crystallographic statistics**

Dataset:	LasR LBD L130F:3OC <sub>14</sub> HSL	LasR LBD L130F:3OC <sub>10</sub> HSL
Resolution (Å)	30.0-1.90 (1.98-1.90)	30.0-2.13 (2.20-2.13)
Unique Reflections	25887	14503
Completeness	96.6 (86.0)	96.4 (84.7)
<Redundancy>	2.5 (1.7)	2.0 (1.6)
R <sub>merge</sub>	0.102 (0.379)	0.076 (0.664)
< I > / < sigma >	14.4 (1.1)	17.4 (1.4)
Total atoms	2742	2669
R <sub>work</sub> (%)	22.1 (30.1)	19.4 (22.9)
R <sub>free</sub> (%)	26.0 (33.0)	25.5 (30.4)
Average B-factor	29.0	43.0
<b>R.m.s. deviation from ideality</b>		
Bond lengths (Å)	0.009	0.009
Bond angles (°)	1.26	1.02
Dihedral angles (°)	17.3	11.1
Phi-Psi values (Ramachandran)		
Most favored		
Additionally allowed		
Outliers		

**Appendix Table 2: Primers used in this study**

Name	Sequence	Description
ARM203	cggtttcttgagctggaacgc	LasR forward primer
ARM204	aacggccataatggccgctac	LasR reverse primer
ARM357	acgctgagggtcagcgcgccgag	lasRS129T forward
ARM358	ctcggcgcgctgaccctcagcgt	lasRS129T reverse
ARM371	cacgctgaggcacagcgcgccga	lasRS129C forward
ARM372	tcggcgcgctgtgcctcagcgtg	lasRS129C reverse
ARM375	ctccacgctgaggaacagcgcgccgagtt	lasRS129F forward
ARM376	aactcggcgcgctgttctcagcgtggaag	lasRS129F reverse
ARM425	ctccacgctgagccacagcgcgccgagt	lasRS129W forward
ARM426	actcggcgcgctgtggctcagcgtggaag	lasRS129W reverse
ARM437	ctccacgctgaagctcagcgcgcc	lasRL130F forward
ARM438	ggcgcgctgagcttcagcgtggaag	lasRL130F reverse
ARM441	ctcggcgcgctgatgctcagcgtggaag	lasRS129M forward
ARM442	ctccacgctgagcatcagcgcgccgag	lasRS129M reverse
ARM455	taattaagcttccgaactggaaaagtggtatgtcgcc	lasR pEXG2 upstream forward HindIII
ARM456	tattagtcgacgctcgccgacctgagaggcaaga	lasR pEXG2 downstream reverse Sall
ARM470	taattgatccccgaactggaaaagtggtatgtcgcc	pUCP18 lasR upstream forward BamHI
ARM471	tattagaattcgtcgccgacctgagaggcaaga	pUCP18 lasR downstream reverse EcoRI
ARM472	ctcgactaaccagatgccc	pUCP18 lasR overlapping forward
ARM473	ttggagcgaacgacctacac	pUCP18 lasR overlapping reverse
ARM516	tccttaggcaacagggcgaagaggatcttcg	lasRG38A forward

ARM517	cgaagatcctcttcgccctgttgccctaagga	lasRG38A reverse
ARM518	gctgtccttaggcaacaggatgaagaggatcttcgagaat	lasRG38I forward
ARM519	attctcgaagatcctcttcacctgttgccctaaggacagc	lasRG38I reverse
ARM520	tggctgtccttaggcaacagtaggaagaggatcttcgagaatc	lasRG38L forward
ARM521	gattctcgaagatcctcttcctactgttgccctaaggacagcca	lasRG38L reverse

**Appendix Table 3: Strains used in this study**

Name	Description	Source
UCBPP-PA14	Wild type, generous gift from Dr. George O'Toole	(Kukavica-Ibruli et al., 2008)
SM51	<i>P. aeruginosa</i> $\Delta lasI$	(Mukherjee et al., 2017)
JP113	<i>E. coli</i> pBad-A- <i>lasR</i> PA14 pCS26- <i>lasB-lux</i>	(Paczkowski et al., 2017)
AM05	<i>E. coli</i> pBad-A- <i>lasR</i> -S129F pCS26- <i>lasB-lux</i>	This study
AM06	<i>E. coli</i> pBad-A- <i>lasR</i> -S129C pCS26- <i>lasB-lux</i>	This study
AM31	<i>E. coli</i> pBad-A- <i>lasR</i> -S129M pCS26- <i>lasB-lux</i>	This study
AM32	<i>E. coli</i> pBad-A- <i>lasR</i> -S129T pCS26- <i>lasB-lux</i>	This study
AM28	<i>E. coli</i> pBad-A- <i>lasR</i> -S129W pCS26- <i>lasB-lux</i>	This study
AM29	<i>E. coli</i> pBad-A- <i>lasR</i> -L130F pCS26- <i>lasB-lux</i>	This study
AM30	<i>E. coli</i> pBad-A- <i>lasR</i> -L128F pCS26- <i>lasB-lux</i>	This study
AM51	<i>E. coli</i> pBad-A- <i>lasR</i> -Y47R pCS26- <i>lasB-lux</i>	This study
AM53	<i>E. coli</i> pBad-A- <i>lasR</i> -Y47S pCS26- <i>lasB-lux</i>	This study
AM59	<i>E. coli</i> pBad-A- <i>lasR</i> -G38A pCS26- <i>lasB-lux</i>	This study
AM60	<i>E. coli</i> pBad-A- <i>lasR</i> -G38I pCS26- <i>lasB-lux</i>	This study
AM61	<i>E. coli</i> pBad-A- <i>lasR</i> -G38L pCS26- <i>lasB-lux</i>	This study
AM41	<i>P. aeruginosa</i> $\Delta lasI$ <i>lasR</i> - S129F	This study
AM43	<i>P. aeruginosa</i> $\Delta lasI$ <i>lasR</i> - L130F	This study

AM57	<i>P. aeruginosa</i> $\Delta lasI$ $\Delta rsaL$	This study
pJP14	<i>E. coli</i> DH-IBP-8xHis- <i>lasR:LBD</i> (1-170)	(Paczkowski et al., 2017)
AM58	<i>E. coli</i> DH-IBP-8xHis- <i>lasR:LBD-L130F</i> (1-170)	This study
SM10 pir	<i>E. coli thi thr leu tonA lacY</i> <i>supE recA::RP4-2-Tc::Mu</i>	(Simon et al., 1983)
pEXG2	Allelic exchange vector with pBR origin, gentamicin resistance, <i>sacB</i> , generous gift from Dr. Joseph Mougous	(Borlee et al., 2010)
pUCP18	<i>E. coli-Pseudomonas</i> Amp <sup>r</sup> shuttle vector	(Schweizer., 1991)
BL21 (DE3)	<i>E. coli</i> B F <sup>-</sup> <i>dcm ompT hsdS(r - m -)</i> <i>gal</i> $\lambda$ (DE3)	Agilent
One Shot Top10	<i>E. coli</i> F- <i>mcrA</i> $\Delta(mrr-hsdRMS-mcrBC)$ $\phi 80/lacZ\Delta M15$ $\Delta lacX74$ <i>recA1</i> <i>araD139</i> $\Delta(ara-leu)7697$ <i>galU</i> <i>galK</i> <i>rpsL</i> (StrR) <i>endA1</i> <i>nupG</i>	Thermo-Fisher

## Appendix Figure 1: Synthetic schemes

