SUPPORTING INFORMATION

Table S1. NCBI BlastP results for PqsE homologs obtained from the *Pseudomonas* Genome DB[1]. Included in this table are standard BlastP output with *P. aeruginosa* PqsE used as a reference.

Table S2. Structural and sequence alignment table. This table shows the root mean square deviations and TM-score of predicted structures from *P. fluorescens* NCTC 10783, *B. cepacia* ATCC 25416, *B. thailandensis* E264, *B. pseudomallei* K96243 compared to the experimental structure of PqsE from *P. aeruginosa* (PDB ID: 5HIO[2]). This table also includes the percent amino acid identity and protein sequence length of each protein.

Table S3. Strains and plasmids used in this study. This table includes the names, genotypes, antibiotic resistance markers, and sources of all strains used in this study.

Table S4. Oligonucleotides used in this study. This table includes the names, sequences (5' to 3'), and description of each oligonucleotide used in this study.

Supplementary FIG 1. Sequence and structural comparison of PqsE and HhqE orthologs. (a) Multiple-sequence alignment of full-length protein sequences of PqsE from *P. aeruginosa* PA14 and *P. fluorescens* NCTC 10783 or HhqE in *B. cepacia* ATCC 25416, *B. pseudomallei* K96243, and *B. thailandensis* E264. Active site residues H69, H71, D73, and H74 (numbering according to PA14 PqsE) are highlighted in red. Residues involved in PqsE dimerization are highlighted in blue; residues unique to PqsE that are required for the PqsE-RhlR interaction are highlighted in green. Secondary structure assignments are represented above the sequence, determined from the PA14 PqsE experimental structure (PDB: 7KGW[3]). (b) Structural overlay of PA14 PqsE (PDB: 7KGW[3]) with predicted structures of the *Pseudomonas* and *Burkholderia* orthologs. For clarity, structures are depicted as ribbons, with the color scheme corresponding to the alignment in (a). All structure images were generated in ChimeraX[4-6].

Supplementary FIG 2. Estimation of PqsE, PqsE^{NI} and HhqE^{Bc} molecular weights by size exclusion chromatography. The peak elution profiles for PqsE, PqsE^{NI} and HhqE^{Bc} proteins were compared to peak elution volumes of MW protein standards: Vitamin B12 (1.35 kDa, V_e = 21.68 mL), myoglobin (17 kDa, V_e = 20.50 mL), ovalbumin (44 kDa, V_e = 16.49 mL), γ-globulin (158 kDa, V_e = 12.68), and thyroglobulin (670 kDa, V_e = 9.01). V_e indicates the peak elution volume. PqsE, PqsE^{NI} and HhqE^{Bc} had a V_e of 14.30 mL, 16.10 mL, and 16.45 mL respectively. The void volume (V_o) was determined at 8.08 mL as the elution volume of thyroglobulin. A standard curve was generated from a linear fit of the $\log_{10}$ MW (Da) of the protein standards versus the elution parameter K_{av} where $K_{av} = (V_e-V_o)/(V_c-V_o)$. V_c is equal to the total column volume. The MWs of PqsE, PqsE^{NI}, and HhqE^{Bc} were estimated to be 79 kDa, 34 kDa, and 32 kDa based on a linear fit to the MW protein standards.

Supplementary FIG 3. MassFludix HC experiments of PqsE, PqsE^{NI}, and HhqE^{Bc}. Mass photometry measurements of PqsE (A), PqsE^{NI} (B), and HhqE^{Bc} (C) following rapid dilution from 50 uM to 50 nM using the MassFluidix HC system (Refeyn). The number of counts is shown as a function of molecular weight (kDa). Mirror peaks in panels (B) and (C) arise due to the instrument's detection limit (50 kDa), reflecting PqsE^{NI}, and HhqE^{Bc}, the presence of which are too small to be directly measured.

Supplementary FIG 4. Structural similarities of LuxR-type receptors. Structural overlay of AlphaFold-Multimer predicted structures of CepR from *B. cepacia* and PmlR from *B. pseudomallei* compared to the experimental structure of RhlR (PDB ID: 8DQ0[7]). The percentage amino acid identity and root mean square deviation (rmsd) values of CepR and PmlR relative to PA14 RhlR are indicated. The inset highlights the universally conserved YXXXW motif within the ligand binding pocket (LBP) of LuxR-type receptors (numbering based on PA14 RhlR). The solvent-accessible volume of the RhlR LBP, shown in light blue is included to provide structural context. All structure images were generated in ChimeraX.

Supplementary FIG 5. HhqE does not complement pyocyanin production in a Δ*pqsE* strain of *P. aeruginosa*. (a) Pyocyanin production in Δ*pqsE* strains of *P. aeruginosa* expressing plasmid-borne *pqsE* or *hhqE*^{Bc} was quantified by OD_{695}/OD_{600} and LC-MS. OD_{695}/OD_{600} values were normalized to levels observed in the strain expressing WT *pqsE* (center bar). (b) Growth of Δ*pqsE* strains carrying plasmids as in (a) on LB agar. Complementation of pyocyanin production was observed with WT *pqsE* but not with *hhqE* from *B. cepacia*.

Supplementary FIG 6. HhqE does not enhance CepR binding to promoter DNA. (a) EMSA analysis of *cepI* promoter DNA alone (minus symbol, left lane), with increasing concentrations of purified CepR:C_8HSL (left half), and with both CepR and HhqE (right half). An EMSA using *rhlA* promoter DNA was included as a negative control (middle). A representative SDS-PAGE gel shows protein levels for each sample (bottom). Final concentrations of CepR and HhqE were: 0 nm, 280 nM, 420 nM, 630 nM, 950 nM, and 1,350 nM.

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virulence factor production in *Pseudomonas aeruginosa*. *Microbiology spectrum*, 10.1:e02108-21. https://doi.org/10.1128/spectrum.02108-21.

Figure S1

A

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                  β1        β2         β3              α1
P. aeruginosa     MLRLSAPGQLDDDLCLLGDVQVPVFLLRLGEASWALVEGGISRDAELVWADLCRWVAD--   58
P. fluorescens    MLRLSAPGQLDDDLCLLGDVQVPVFLLRLGEASWALVEGGISRDAELVWADLCRWVAD--   58
B. cepacia        -MLNASPGWIEPRLALIGTADVPLYVIVNDE-AATLIEGGLSGMTALVWQQLHDLLRDFG   58
B. pseudomallei   -MQHGTPGRIDSRLAIVGTADVPLYVIVNDE-AATLVEGGLSGMTELVWRQLHELLSDYG   58
B. thailandensis  -MQHSTPGWIDSQLAIVGTADVPLYVVVNDE-AATLVEGGLSGMTGLVWRQLHDLLKDYG   58

                     β4          α2              β5      α3         α4
P. aeruginosa     -PSQVHYWLITHKHYDHCGLLPYLCPRLPNVQVLASERTCQAWKSESAVRVVERLNRQLL   117
P. fluorescens    -PSQVHYWLITHKHYDHCGLLPYLCPRLPNVQVLASERTCQAWKSESAVRVVERLNRQLL   117
B. cepacia        GIRHLRYWLITHSHYDHCSLLMTLKSRMPWLHVSGSPDAFDAFQSPSACRTIRQLDEHAS   118
B. pseudomallei   GIRHLRYWLITHSHYDHCSLLGTLAPRMPWLHIVGSAATADALQSPSACRTIRALDAQAC   118
B. thailandensis  GIRHLRYWLITHSHYDHCSLLGTLAPRMPWLHIVGSPETADALQSPSARRTIRKLDAQAS   118

                                     β6              β7           β8
P. aeruginosa     RAEQRLP--EACAWDALPVRAVADGEWLELGPRHRLQVIEAHGHSDDHVVFYDVRRRRLF   175
P. fluorescens    RAEQRLP--EACAWDALPVRAVADGEWLELGPRHRLQVIEAHGHSDDHVVFYDVRRRRLF   175
B. cepacia        RSWEPAAGADFTELSDLPFYPVNPGAQLDIGDGMQIRTIALPGHSRCQFGYYCPQLDIGF   178
B. pseudomallei   AAWEPVAVA--AELSDIPLYPLNPGRSLDIGEGMRMRALALPGHSACLLGYHCPQLDLLF   176
B. thailandensis  DAWEPVADADLADLSDIPLYPLNPGRALDIGEGMRMRAVALPGHSACLLGYHCPQLDLLF   178

                      β9                    α5           β10
P. aeruginosa     CGDALGEFDEAEGVWRPLVFDDMEAYLESLERLQRLPTLLQLIPGHGGLLRGRLAADGAE   235
P. fluorescens    CGDALGEFDEAEGVWRPLVFDDMEAYLESLERLQRLPTLLQLIPGHGGLLRGRLAADGAE   235
B. cepacia        VSDALGEFQDA-THWLPLVFQDLFAYRHSLDVIEQLHAP-RLALGHHGILTGELARSAAR   236
B. pseudomallei   VSDALGEYHAP-TQWLPLVFQDLAAYRLSLDAIERQHAS-RIALGHHGIVGGGIARHAVR   234
B. thailandensis  VSDALGEYHAP-TQWLPLVFQDLPAYRQSLDEIERRHAS-RIALGHHGIVGGDVARHAVR   236

                            α6                  α7                  α8
P. aeruginosa     SAYTECLRLCRRLLWRQSM--GESLDELSEELHRAWGGQSVDFLPGELHLGSMRRMLEIL   293
P. fluorescens    SAYTECLRLCRRLLWRQSM--GESLDELSEELHRAWGGQSVDFLPGELHLRSMRRMLEIL   293
B. cepacia        HAR-ACLDAREA-DARAVRGNAAATQALAHQWTARYAARSEKVVPRFLHLKSMTHMIDLF   294
B. pseudomallei   HAR-DGLAARDD-EARAARGDANATRALARQWTERYAARSANVVPRALHLKSMERMIDLF   292
B. thailandensis  HAR-DGLAARGD-EARAASGDANATRALAQQWTERYAARSEKVVPRALHLKSMERMIDLF   294

P. aeruginosa     SRQALPLD   301
P. fluorescens    SRQALPLD   301
B. cepacia        HRAE----   298
B. pseudomallei   QRAA----   296
B. thailandensis  QRAA----   298
```

B

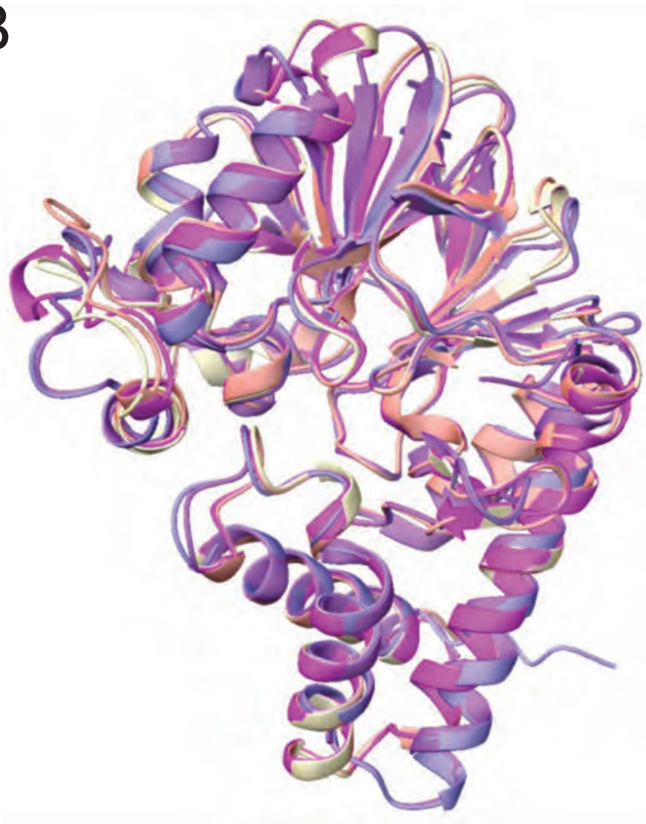
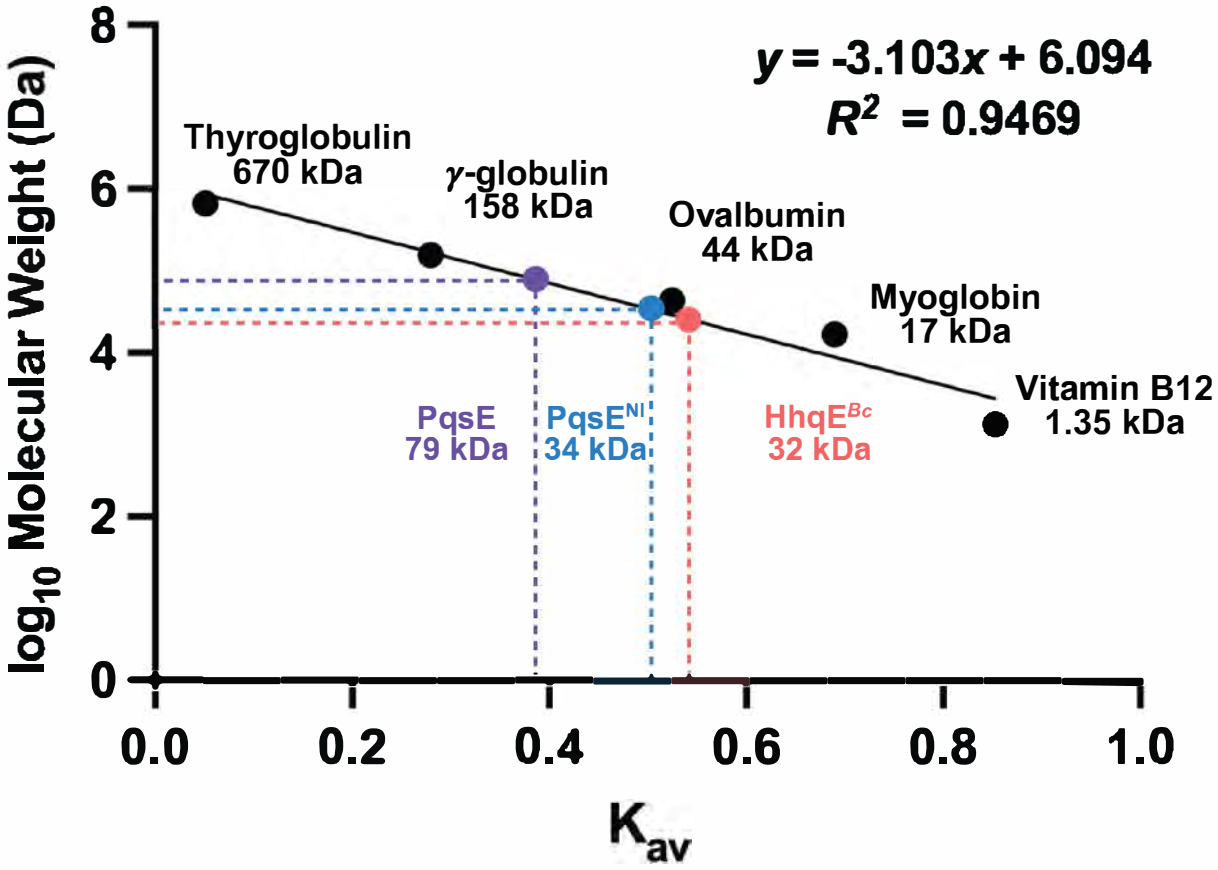


Figure S2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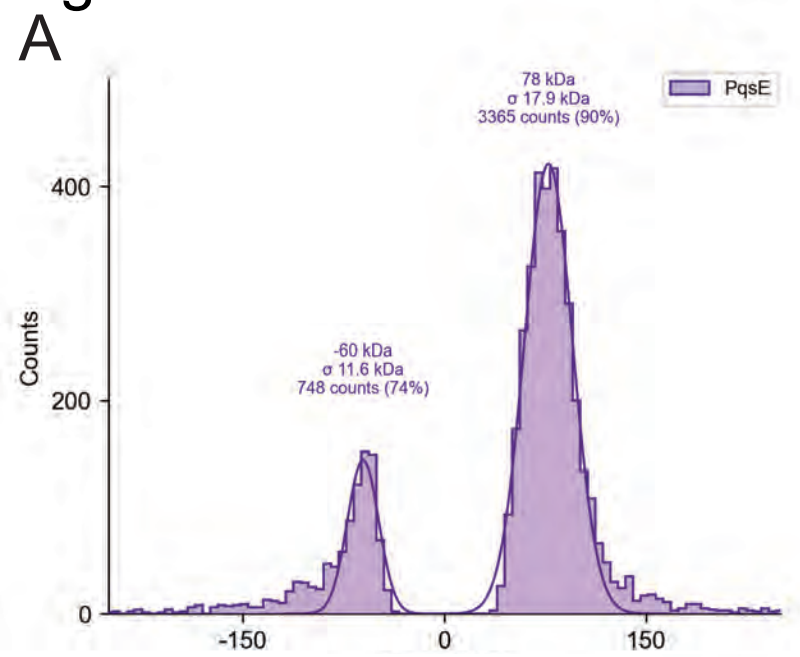
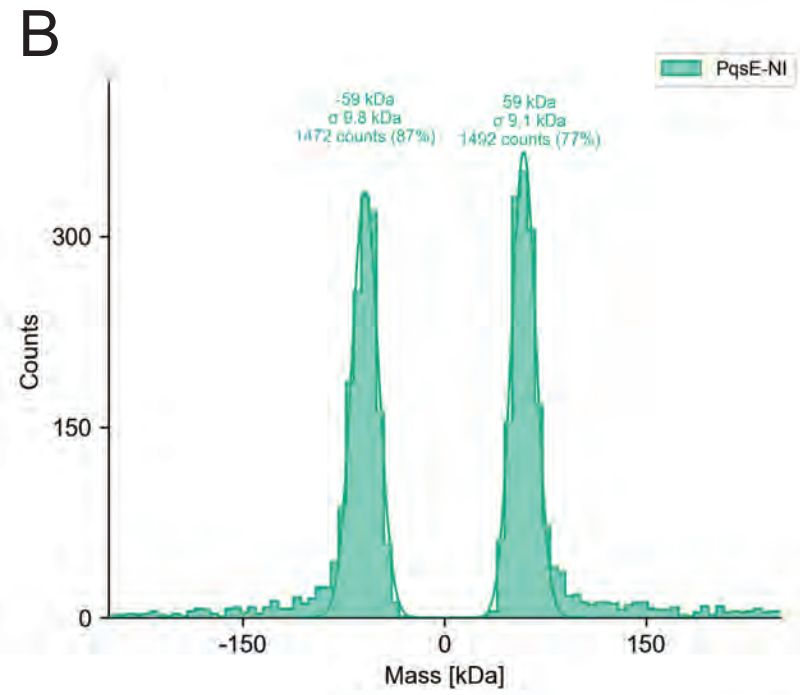
R2 = 0.9469
Thyroglobulin
670 kDa
γ-globulin
158 kDa
Ovalbumin
44 kDa
Myoglobin
17 kDa
Vitamin B12
1.35 kDa
PqsE
79 kDa
PqsENI
34 kDa
HhqEBc
32 kDa
log10 Molecular Weight (Da)
Kav
0
2
4
6
8
0.0
0.2
0.4
0.6
0.8
1.0

Figure S3


A
78 kDa
σ 17.9 kDa
3365 counts (90%)
PqsE
-60 kDa
σ 11.6 kDa
748 counts (74%)
Counts
400
200
0
-150
0
150


B
PqsE-NI
-59 kDa
σ 9.8 kDa
1472 counts (87%)
59 kDa
σ 9.1 kDa
1492 counts (77%)
Counts
300
150
0
-150
0
150
Mass [kDa]

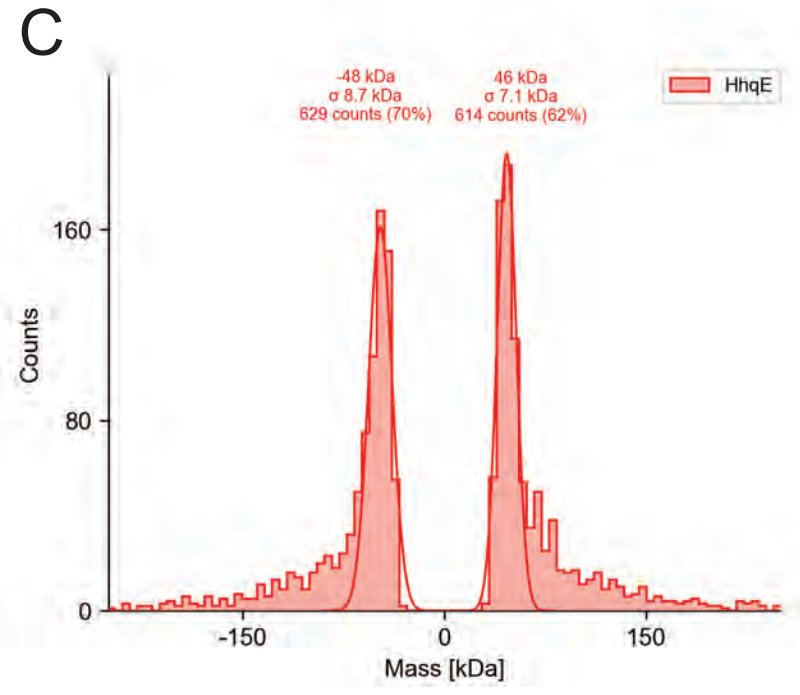

C
-48 kDa
σ 8.7 kDa
629 counts (70%)
46 kDa
σ 7.1 kDa
614 counts (62%)
HhqE
Counts
160
80
0
-150
0
150
Mass [kDa]

Figure S4

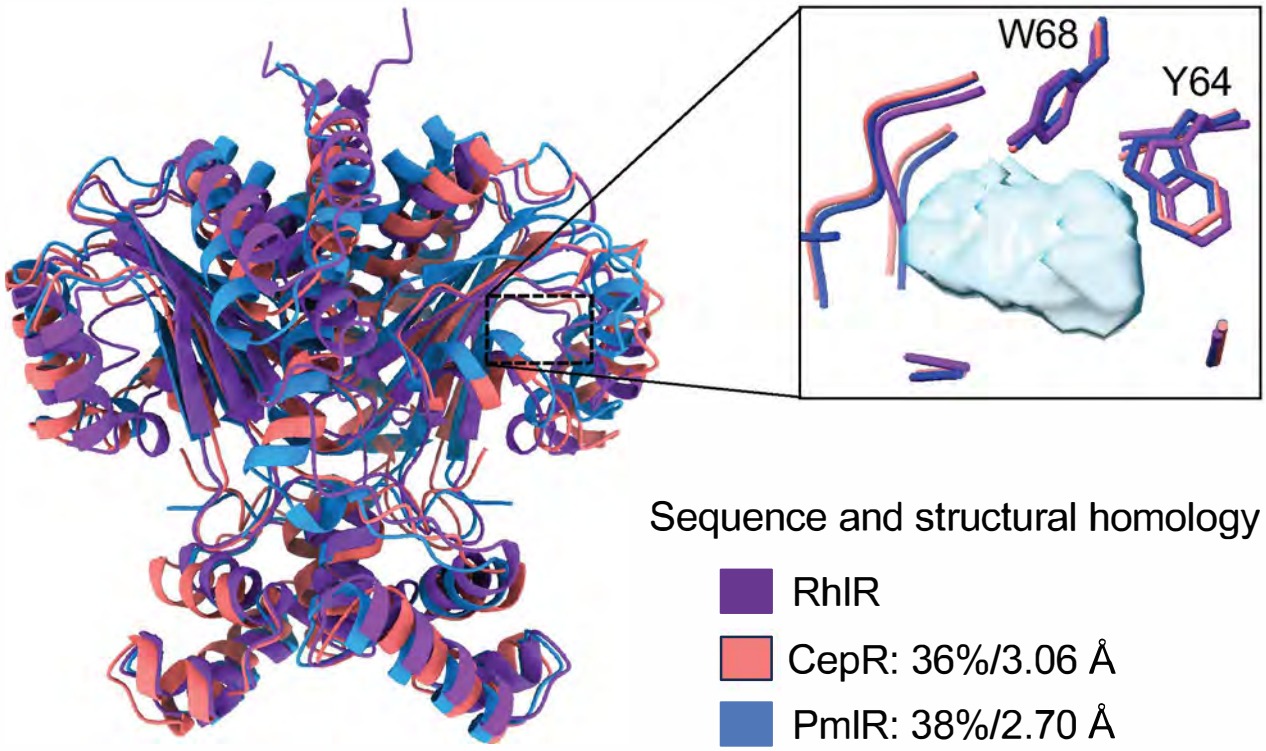
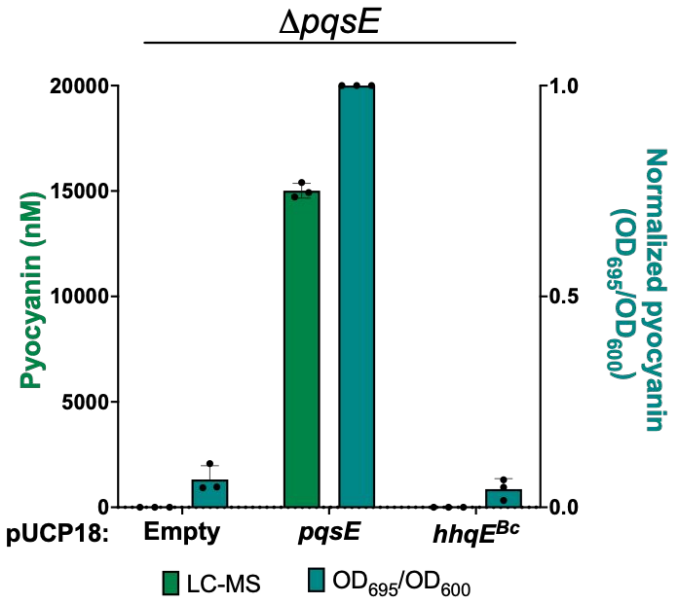

W68
Y64
Sequence and structural homology
RhlR
CepR: 36%/3.06 Å
PmlR: 38%/2.70 Å

Figure S5


ΔpqsE
Pyocyanin (nM)
20000
15000
10000
5000
0
Normalized pyocyanin (OD695/OD600)
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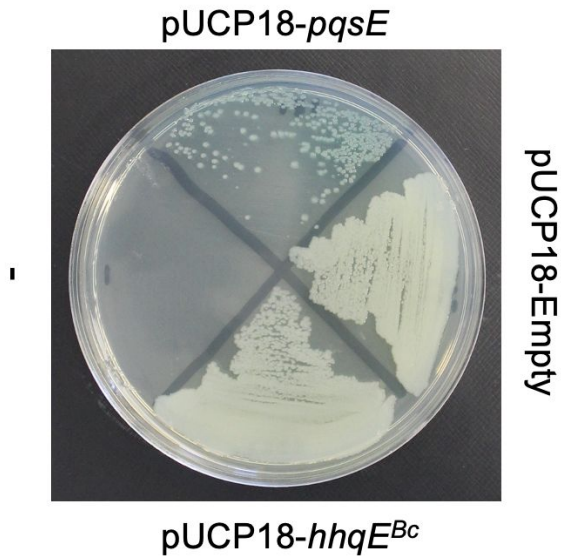
0.5
0.0
pUCP18:
Empty
pqsE
hhqEBc
LC-MS
OD695/OD600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pUCP18-Empty
pUCP18-hhqEBc

Figure S6