Tab. S1: Oligonucleotides used in this study

Oligonucleotide	Sequence 5' → 3'	Purpose
PrM_036_XTEN30-F	ACACCAGAATCAGGTCCAGGGACGAGCACTGAACCTTCTGAAGGATCGGCACCAATGAGGAATGACGGAGGCTTTTTG	Forward PCR Primer to introduce a 30 residue XTEN-Linker between PqsE and RhlR
PrM_037_XTEN30-R	CCTGATTCTGGTGTTGCGGACTCGCTTGTTCCTGGTGTCTCACTTCCAGAGTCCAGAGGCAGCGCCTG	Reverse PCR Primer to introduce a 30 residue XTEN-Linker between PqsE and RhlR
PrM_044_R150A-F	CGGCATGCACTGCAGGTCATAGAGGCCC	Forward SDM Primer to introduce R150A Substition in PqsE
PrM_045_R150A-R	CTGCAGTGCATGCCGCGGTCCCAG	Reverse SDM Primer to introduce R150A Substition in PqsE
PrM_046-R170A-F	GACGTGGCACGCCGCCGCCTGTTCTG	Forward SDM Primer to introduce R170A Substition in PqsE
PrM_047-R170A-R	GCGGCGTGCCACGTCGTAGAAAACCACG	Reverse SDM Primer to introduce R170 Substition in PqsE
PrM_048-R172A-F	CGACGTGCCCGCCTGTTCTGCGGC	Forward SDM Primer to introduce R172A Substition in PqsE
PrM_049-R172A-R	CAGGCGGGCACGTCGCACGTCGTAGAAAACC	Reverse SDM Primer to introduce R172A Substition in PqsE
PrM_050-E206A-F	TCCCTGGCACGTCTGCAGCGTCTGC	Forward SDM Primer to introduce E206A Substition in PqsE
PrM_051-E206A-R	CAGACGTGCCAGGGACTCCAGGTAAGCC	Reverse SDM Primer to introduce E206A Substition in PqsE
PrM_052-Q209A-F	CGTCTGGCACGTCTGCCGACCCTGC	Forward SDM Primer to introduce Q209A Substition in PqsE
PrM_053-Q209A-R	CAGACGTGCCAGACGTTCCAGGGACTCC	Reverse SDM Primer to introduce Q209A Substition in PqsE
PrM_054-R210A-F	CTGCAGGCACTGCCGACCCTGCTGC	Forward SDM Primer to introduce R210A Substition in PqsE
PrM_055-R210A-R	CGGCAGTGCCTGCAGACGTTCCAGGGAC	Reverse SDM Primer to introduce R210A Substition in PqsE

PrM_058-R148A-F	CCGGCACATCGCCTGCAGGTCATAGAG	Forward SDM Primer to introduce R148A Substition in PqsE
PrM_059-R148A-R	CAGGCGATGTGCCGGTCCCAGCTCCAGCC	Reverse SDM Primer to introduce R148A Substition in PqsE
Pr029_PqsE-Duet_F	CTTTAAGAAGGAGATATACCATGGGCCATCATCATCATCATCA	Forward Primer to amplify pqsE sequence
Pr030_PqsE-Duet_R	TACGATTACTTTCTGTTCGATCAGTCCAGAGGCAGCG	Reverse Primer to amplify pqsE sequence
Pr031_Duet_Frag_F	TCGAACAGAAAGTAATCGTATTGTACACG	Forward Primer to amplify the region between the two MCS in pET-Duet-1 sequence
Pr032_Duet_Frag_R	ATGTATATCTCCTTCTTATACTTAACTAATATACTAAGATGGGGAATTG	Reverse Primer to amplify the region between the two MCS in pET-Duet-1 sequence
Pr033_RhlR_F	TATAAGAAGGAGATATACATATGAGGAATGACGGAGGCTTTT	Forward Primer to amplify rhlR sequence
Pr034_RhlR_R	TTTACCAGACTCGAGGGTACTCAGATGAGACCCAGCGCC	Reverse Primer to amplify rhlR sequence
Pr039_RhlR_500-F	CGGGGATCCTCTAGAGTCGACGCAGCGCGCCTACGC	Forward Primer to amplify the 500 bp upstream region of rhlR
Pr041-RhlR-Prom-R	CATTGCAGTAAGCCCTGATCGATAAAATGCATC	Reverse Primer to amplify the 500 bp upstream region of rhlR
Pr042-RhlR-F	ATCGATCAGGGCTTACTGCAATGAGGAATGACGGAGGCTTTTTG	Forward Primer to amplify the rhlR sequence for cloning in pUCP24m
Pr036_His_RhlR_R	ACGACGGCCAGTGCCAAGCTTCAGATGAGACCCAGCGCC	Reverse Primer to amplify the rhlR sequence for cloning in pUCP24m
Pr043-RhlR-75-F	GATCAGGGCTTACTGCAATGCGCAACGATGGCGGCTTTC	Forward Primer to amplify the rhlR-PROSS mutant sequences for cloning in pUCP24m
Pr038_His_RhlR_P75_R	AAACGACGGCCAGTGCCAAGCTTAAATCAGGCCCAGCGCCG	Reverse Primer to amplify the rhlR-PROSS mutant sequences for cloning in pUCP24m
PqsE_E187R_for	GGGCGAGTTCGACGAGGCACGTGGGGTGTGGCGGCCGCTGG	Forward QuikChange Primer to introduce the named mutations into PqsE
PqsE_E187R_rev	CCAGCGGCCGCCACACCCCACGTGCCTCGTCGAACTCGCCC	Reverse QuikChange Primer to introduce the named mutations into PqsE

RhlRwt_PstI	AAACTGCAGTCCAACCCGGTCTGCCTGA	Forward PCR Primer to amplify RhlR WT DBD with PstI restriction site
RhlRwt_KpnI_rv	AAAGGTACCTCAGATGAGACCCAGCGCCGC	Reverse PCR Primer to amplify RhlR WT DBD with KpnI restriction site
RP75_S173MQ175M_fw	CGAACTGAACCATCCGATGCTGATGTCCAACCCGGTCTGCC	Forward QuikChange Primer to introduce the named mutations into RhlR-P75
RP75_S173MQ175M_rv	GGCAGACCGGGTTGGACATCAGCATCGGATGGTTCAGTTCG	Reverse QuikChange Primer to introduce the named mutations into RhlR-P75
RhlR_WT_R37A_fw	GGAAAAGGAAGTGCGGGCCCTGGGCTTCGATTAC	Forward QuikChange Primer to introduce the named mutation into RhlR WT
RhlR_WT_R37A_rv	GTAATCGAAGCCCAGGGCCCGCACTTCCTTTTCC	Reverse QuikChange Primer to introduce the named mutation into RhlR WT
RhlR_WT_D41A_fw	GCGCCTGGGCTTCGCTTACTACGCCTATG	Forward QuikChange Primer to introduce the named mutation into RhlR WT
RhlR_WT_D41A_rv	CATAGGCGTAGTAAGCGAAGCCCAGGCGC	Reverse QuikChange Primer to introduce the named mutation into RhlR WT
RhlR_WT_E147A_fw	CATCTCCAGCTTCGCGCGCGAGGAAATCC	Forward QuikChange Primer to introduce the named mutation into RhlR WT
RhlR_WT_E147A_rv	GGATTTCCTCGCGCGCGAAGCTGGAGATG	Reverse QuikChange Primer to introduce the named mutation into RhlR WT
RhlR_WT_E150A_fw	TCGAGCGCGAGGCAATCCGCCTGCG	Forward QuikChange Primer to introduce the named mutation into RhlR WT
RhlR_WT_E150A_rv	CGCAGGCGGATTGCCTCGCGCTCGA	Reverse QuikChange Primer to introduce the named mutation into RhlR WT
RhlR_WT_R36A_fw	TGGAAAAGGAAGTGGCGCGCCTGGGCTTCG	Forward QuikChange Primer to introduce the named mutation into RhlR WT
RhlR_WT_R36A_rv	CGAAGCCCAGGCGCGCCACTTCCTTTTCCA	Reverse QuikChange Primer to introduce the named mutation into RhlR WT
RhlR_WT_Q140AQ141A_fw	TTTCCGTGGCGCGCGACGCGGCGAACATCTCCAGCTTCG	Forward QuikChange Primer to introduce the named mutations into RhlR WT

RhlR_WT_Q140AQ141A_rv	CGAAGCTGGAGATGTTCGCCGCGTCGCGCGCCACGGAAA	Reverse QuikChange Primer to introduce the named mutations into RhlR WT
RWT_R154A_fw	GAGGAAATCCGCCTGGCGCTGCGTTGCATGA	Forward QuikChange Primer to introduce the named mutation into RhlR WT
RWT_R154A_rv	TCATGCAACGCAGCGCCAGGCGGATTTCCTC	Reverse QuikChange Primer to introduce the named mutation into RhlR WT
*pqsE*_up_F	CGAATTCGGCGAGAGTCTCGAAGACG	Forward PCR primer for the amplification of upstream *pqsE* sequence (EcoRI site underlined)
*pqsE*_up_R	CAGAGGCAGCGAAAGCCTCAACATGGC	Reverse PCR primer for the amplification of upstream *pqsE* sequence
*pqsE*_dw_F	AGGCTTTCGCTGCCTCTGGACTGAGGC	Forward PCR primer for the amplification of downstream *pqsE* sequence
*pqsE*_dw_R	CGGATCCCTGCGACTCTTCCTGGGG	Reverse PCR primer for the amplification of downstream *pqsE* sequence (BamHI site underlined)
pqsE_F	CAACCTGTCGATCCTGCTCG	Forward primer for the verification of the *pqsE* deletion
pqsE_R	CAGGCTGGACAGGCCATG	Reverse primer for the verification of the *pqsE* deletion
rhlR_Up_F	TATGCGGCCGCTGCAGCGCGCCTACGCG	Forward PCR primer for the amplification of upstream *rhlR* sequence (NotI site underlined)
rhlR_Up_R	TCAGTCAGTCAGCTAGCTGCAGTAAGCCCTGATCGATAAAATGCA	Reverse PCR primer for the amplification of upstream *rhlR* sequence (NheI site underlined)
rhlR_Dw_F	GCTAGCTGACTGACTGAAGCGCAGGGCGCGCCG	Forward PCR primer for the amplification of upstream *rhlR* sequence (NheI site underlined)
rhlR_Dw_R	TATAAGCTTGGCGGCGTAGCGCGAAAGC	Reverse PCR primer for the amplification of downstream *rhlR* sequence (HindIII site underlined)
*rhlR*_F	CTGTCGGCGTTTCATGGAATTG	Forward primer for the verification of the *rhlR* deletion

*rhlR*_R	AAGACTTGATGCCGGTAGCGTC	Reverse primer for the verification of the *rhlR* deletion
Sp_*rhlI*_F	gcgcgCGAATTGCTCTCTGAATCGC	Sense-oriented oligonucleotide to construct the specific *rhlI* spacer (protruding end indicated in lowercase)
Sp_*rhlI*_R	aaacGCGATTCAGAGAGCAATTCGc	Antisense-oriented oligonucleotide to construct the specific *rhlI* spacer (protruding end indicated in lowercase)
*rhlI*_ ssDNA_R	CATGCGGCAGGAGAAGCGGAAAAA AGTGCGCGAAACGGCTGACGACC*TC A***CAT**GACCAAGTCCCCGTGTCGTGC CGGCCGAGAAAAAAAAGGCGGCAT CC	Recombineeting mutagenic oligonucleotide to generate the clean deletion of *rhlI* (Start codon bold and Stop codon in italic)
*rhlI*_F	TCCTCCTTTAGTCTTCCCCC	Forward primer for the verification of the *rhlI* deletion
*rhlI*_R	GAGAGACTACGCAAGTCGGC	Reverse primer for the verification of the *rhlI* deletion

Primers were ordered from either Eurofins Genomics or Sigma Aldrich.

Tab. S2: Plasmids used in this study

Plasmid	Features / Function	Supplier / Source
pET-Duet-1		Novagen
pUCP24m	pUCP24 Vector with an introduced stop codon in the lacZα-peptide	kindly provided by Prof. D. Jahn
pUCP24m His-PqsE WT	pUCP24m derivative encoding the sequence for PqsE with a N-terminal His-Tag	this study
pUCP24m His-PqsE-R148A	pUCP24m derivative encoding the sequence for PqsE-R148A with a N-terminal His-Tag	this study
pUCP24m His-PqsE-R150A	pUCP24m derivative encoding the sequence for PqsE-R150A with a N-terminal His-Tag	this study
pUCP24m His-PqsE-R170A	pUCP24m derivative encoding the sequence for PqsE-R170A with a N-terminal His-Tag	this study
pUCP24m His-PqsE-R172A	pUCP24m derivative encoding the sequence for PqsE-R172A with a N-terminal His-Tag	this study
pUCP24m His-PqsE-E187R	pUCP24m derivative encoding the sequence for PqsE-E187R with a N-terminal His-Tag	this study
pUCP24m His-PqsE-E206A	pUCP24m derivative encoding the sequence for PqsE-E206A with a N-terminal His-Tag	this study
pUCP24m His-PqsE-Q209A	pUCP24m derivative encoding the sequence for PqsE-Q209A with a N-terminal His-Tag	this study
pUCP24m His-PqsE-R210A	pUCP24m derivative encoding the sequence for PqsE-R210A with a N-terminal His-Tag	this study
pUCP24m His-PqsE-XTEN30-RhlR	pUCP24m derivative encoding the sequence for PqsE and RhlR linked by a 30 residue XTEN Linker and a N-terminal His-Tag	this study
pUCP24m prhlR RhlR WT	pUCP24m derivative encoding the wildtype RhlR sequence and 500 bp of the region upstream of RhlR	this study
pUCP24m prhlR RhlR R37A	pUCP24m derivative encoding the RhlR-R37A sequence and 500 bp of the region upstream of RhlR	this study
pUCP24m prhlR RhlR D41A	pUCP24m derivative encoding the RhlR-D41A sequence and	this study

	500 bp of the region upstream of RhlR	
pUCP24m prhlR RhlR E147A	pUCP24m derivative encoding the RhlR-E147A sequence and 500 bp of the region upstream of RhlR	this study
pUCP24m prhlR RhlR E150A	pUCP24m derivative encoding the RhlR-E150A sequence and 500 bp of the region upstream of RhlR	this study
pUCP24m prhlR RhlR R154A	pUCP24m derivative encoding the RhlR-R154A sequence and 500 bp of the region upstream of RhlR	this study
pUCP24m prhlR RhlR Q140A Q141A	pUCP24m derivative encoding the RhlR-Q140A Q141A sequence and 500 bp of the region upstream of RhlR	this study
pUCP24m prhlR RhlR P61	pUCP24m derivative encoding the RhlR-P61 sequence and 500 bp of the region upstream of RhlR	this study
pVP008 Strep-TEV-RhlR-P75	pCOLA Duet (Novagen) derivate with TEV protease cleavage site, RhlR-P75 cloned between NotI/KpnI restriction sites	this study
pVP008 Strep-TEV-RhlR P61	pCOLA Duet (Novagen) derivate with TEV protease cleavage site, RhlR-P61 cloned between NotI/KpnI restriction sites	this study
pET19m His_6-TEV-PqsE	pET19b (Novagen) derivate with TEV protease cleavage site and sequence encoding for PqsE	Zender and Witzgall et al. 2016
pET19m His_6-TEV-PqsE-E187R	pET19b (Novagen) derivate with TEV protease cleavage site and sequence encoding for PqsE-E187R	this study
pET-Duet-1 His-TEV-PqsE x RhlR	pET-Duet-1 (Novagen) derivate with TEV protease cleavage site and sequence encoding for PqsE (MCS1) and RhlR (MCS2)	this study
pET-Duet-1 His-TEV-PqsE-XTEN30-RhlR	pET-Duet-1 (Novagen) derivate with TEV protease cleavage site and sequence encoding for PqsE and RhlR linked by a 30 residue XTEN linker	this study

pET26b RhlR WT	Expression vector for untagged RhlR WT, RhlR-encoding sequence cloned between NdeI/XhoI restriction sites	this study
pET19m His_6-TEV-RhlR WT	pET19b (Novagen) derivate with TEV protease cleavage site and sequence encoding for RhlR	this study
pS448•CsR	Sm^R, *oriV pRO1600/ColE1*, *oriT*, *xyl*S, *Pm*-*cas*9, P_{EM7}-sgRNA	Seupt et al.
pSEVA624-*ssr*	Gm^R, pSEVA624 cloned with the *ssr* recombinase sequence	Seupt et al.
pEX-pqsE	Gene replacement vector for PA14 *pqsE*, Amp^R	This study
pEX18Ap2-*rhlR*	Gene replacement vector for PA14 *rhlR* containing a FRT-Gm cassette, Amp^R	This study
pFLP3	FLP expression vector, sacB*, $oriT^+$, Ap^R Tet^R	Choi, Gaynor et al. 2005
pS448•CsR-*rhlI*	Sm^R, derivative of pS448•CsR carrying a P_{EM7}-*rhlI*-targeting sgRNA	This study

Tab. S3: Strains used in this study

Strain	Features / Genotype	Supplier / Source
Escherichia coli Omnimax 2	F´ {*pro*AB *lac*I^q *lac*ZΔM15 *Tn*10(Tet^R) Δ(*ccdA*B)} *mcr*A Δ(*mrr hsd*RMS-*mcr*BC) Φ80(*lac*Z)ΔM15 Δ(*lac*ZYA-*arg*F)U169 *end*A1 *rec*A1 *sup*E44 *thi*-1 *gyr*A96 *rel*A1 *ton*A *pan*D	Invitrogen
Escherichia coli BL21 Codon+ RIL	*F´ ompT hsdS(rB– mB –) dcm+ Tet^R gal λ(DE3) endA Hte [argU ileY leuW Cam^R]*	Agilent
Pseudomonas aeruginosa PA14	Wildtype	Liberati, Urbach *et al*. 2006
Pseudomonas aeruginosa PA14 *ΔrhlR*	Knockout strain *ΔrhlR*	Seupt *et al*.
Pseudomonas aeruginosa PA14 *ΔpqsE*	Knockout strain *ΔpqsE*	This study
Pseudomonas aeruginosa PA14 *ΔpqsE ΔrhlR*	Knockout strain *ΔpqsE ΔrhlR*	This study
Pseudomonas aeruginosa PA14 *ΔrhlI*	Knockout strain *ΔrhlI*	This Study
Pseudomonas aeruginosa PA14 *ΔpqsE ΔrhlI ΔrhlR*	Knockout strain *ΔpqsE ΔrhlI ΔrhlR*	This study

Pseudomonas aeruginosa PA14 tn*pqsE* pUCP20::His_6-*pqsE*	Transposon insertion in *pqsE* transformed with pUCP20::His_6-*pqsE*	This study
Pseudomonas aeruginosa PA14 Δ20700ΩGm pUCP20::His_6-*flgZ*	Transposon insertion inf flgZ transformed with pUCP20::His_6-*flgZ*	This study

Tab. S4: Buffers used in this study

Buffer	
Lysis Buffer RhlR-P75/P61	50 mM Tris pH 8.0, 500 mM NaCl, 5 mM DTT
Lysis Buffer RhlR WT	50 mM Tris-HCl pH 8.0, 500 mM NaCl, 50 µM mBTL
Lysis Buffer XTEN	50 mM Bicine pH 8.0, 150 mM NaCl, 5% Glycerol, 50 µM mBTL
Lysis Buffer PqsE/RhlR-complex	50 mM BICINE pH 8, 150 mM NaCl, 5 % Glycerol, 20 µM C4-HSL
Lysis Buffer PqsE E187R	50 mM Tris pH 8, 150 mM NaCl, 10 % Glycerol
Elution buffer RhlR-P75/P61	50 mM Tris-HCl pH 8.0, 500 mM NaCl, 5 mM DTT, 5 mM d-Desthiobiotin
Elution Buffer RhlR WT	50 mM Tris-HCl pH 8.0, 500 mM NaCl, 50 µM mBTL, 500 mM Imidazole
Elution buffer XTEN	50 mM Bicine pH 8.0, 150 mM NaCl, 5% Glycerol, 500 mM Imidazol, 50 µM mBTL
Elution buffer PqsE/RhlR-complex	50 mM BICINE pH 8, 150 mM NaCl, 5 % Glycerol, 20 µM C4-HSL, 500 mM Imidazole
Elution Buffer PqsE E187R	50 mM Tris pH 8, 150 mM NaCl, 500 mM Imidazol 10 % Glycerol
Dialysis buffer RhlR-P75/P61	50 mM Tris-HCl pH 8.0, 500 mM NaCl, 5 mM DTT
Dialysis Buffer RhlR WT	50 mM Tris-HCl pH 8.0, 500 mM NaCl, 50 µM mBTL
Ion Exchange buffer PqsE/RhlR-complex	Buffer A: 50 mM Bicine pH 8, 5 % Glycerol, 20 µM C4-HSL Buffer B 50 mM Bicine pH 8, 1 M NaCl, 5% Glycerol, 20µM C4-HSL
SEC buffer RhlR-P75/P61	50 mM Tris-HCl pH 8.0, 500 mM NaCl, 5 mM DTT, 50 µM mBTL
SEC Buffer RhlR WT	50 mM Tris-HCl pH 8.0, 500 mM NaCl, 50 µM mBTL
SEC buffer XTEN	50 mM Bicine pH 8.0, 100 mM NaCl, 5% Glycerol, 50 µM mBTL
SEC buffer PqsE/RhlR-complex	50 mM BICINE pH 8,1, 50 mM NaCl, 5 % Glycerol, 40 µM C4-HSL
SEC Buffer PqsE E187R	50 mM Tris pH 8, 150 mM NaCl, 10 % Glycerol

Tab. S5: Antibodies used in this study

Antibody	Description	Source
α-RhlR	Anti-RhlR polyclonal rabbit IgG, f.c. 20µg/ml	Davids Biotechnologie GmbH
α-RNAP	Anti-RNAP monoclonal rabbit IgG, f.c. 1:2000	Abcam
α-Rabbit-AP	Anti-Rabbit polyclonal goat IgG (Fc), AP-conjugated, f.c. 1:7500	Promega

Tab. S6: Crystallization conditions and cryo-protectants used in this study

	Temperature	Precipitant	Protein concentration	Cryoprotectant
RhlR-P75 (mBTL)	293 K	0.1 M Bis-Tris pH 6.5, 0.2 M NaCl, 25 % (w/v) PEG 3350	3.5 mg/ml	10% (v/v) (*2R,3R*) -(-)-2,3-butanediol
RhlR-P75 (C4-HSL)	293 K	0.2 M NaSCN, 20 % (w/v) PEG 3350	3.5 mg/ml	10% (v/v) (*2R,3R*) -(-)-2,3-butanediol
RhlR-P61 (mBTL)	293 K	50 mM BICINE pH 8.8, 8.6 % (w/v) PEG 2K, 17.1 % (v/v) PEG MME 350	3 mg/ml	10% (v/v) (*2R,3R*) -(-)-2,3-butanediol
PqsE-XTEN$_{30}$-RhlR (mBTL)	293 K	0.1 M NaAcetate pH 4.22, 1.029 M $(NH_4)_2HPO_4$	12 mg/ml	10% (v/v) (*2R,3R*) -(-)-2,3-butanediol
PqsE/RhlR complex (mBTL)	293 K	0.1 M NaAcetate pH 4.91, 0.972 M $(NH_4)_2HPO_4$, 10 mM TCEP pH 7	10 mg/ ml	20% (w/v) glycerol
PqsE-E187R	293 K	0.16 M CaAcetate, 0.08 M NaCacodylate pH 6.5, 14.4% PEG 8000, 20% glycerol	7.5 mg/ ml	10% (v/v) (*2R,3R*) -(-)-2,3-butanediol

All crystallization experiments were performed in Intelli-Plates (MiTeGen) with crystallization droplets of 150 nl protein and 150 precipitant solution equilibrated against 75 µl reservoirs. PqsE-XTEN30-RhlR was crystallized in MRC Maxi 48-well crystallization plates with 2 µl protein and 2 µl reservoir solution equilibrated against 100 µl reservoir.

Tab. S7: Data collection statistics

	RhlR-P75 (mBTL)	RhlR-P75 (C4-HSL)	RhlR-P61 (mBTL)	PqsE-XTEN30-RhlR (mBTL)	PqsE/RhlR complex (mBTL)	PqsE-E187R
PDB entry	7R3G	7R3H	7R3I	7R3E	7R3J	7R3F
Space group	$P6_1$	$P2_12_12_1$	$P4_12_12$	I422	I422	$P2_12_12_1$
Cell dimensions						
a, *b*, *c* (Å)	164.75, 164.75, 40.3	87.98, 91.55, 117.37	85.90, 85.90, 202.59	159.65, 159.65, 288.74	159.26, 159.26, 284.10	45.53, 60.17, 128.26
α, β, γ (°)	90.0, 90.0, 120.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)*	47.57-2.15 (2.23 - 2.15)	72.18-3.49 (3.62-3.49)	79.16-3.1 (3.21 - 3.1)	144.38-3.46 (3.71-3.46)		27.68-1.60 (1.66-1.60)
Max. ellipsoidal resolution (Å)[a] (direction)[b]				3.46 (a*) 3.46 (b*) 4.17 (c*)	3.06 (a*) 3.06 (b*) 3.97 (c*)	
R_{merge}*	0.231 (2.304)	0.385 (1.660)	0.129 (0.846)	0.126 (2.961)		
R_{pim}*	0.0399 (0.418)	0.0635 (0.318)	0.0148 (0.132)	0.025 (0.563)	0.036 (0.541)	0.0384 (0.3157)
CC(1/2)*	0.998 (0.711)	0.997 (0.785)	1 (0.946)	0.999 (0.562)	0.999 (0.591)	0.998 (0.681)
I / σ(I)*	11.1 (1.6)	7.6 (2.1)	18.5 (2.5)	17.5 (1.5)	17.0 (1.6)	13.06 (2.22)
Completeness						
spherical (%)*	100 (100)	100 (100)	100 (100)	79.0 (21.8)	73.8 (24.6)	99.45 (95.72)
ellipsoidal (%)*				94.8 (58.2)	95.0 (74.2)	
Redundancy*	20.1 (19.8)	13.0 (13.7)	25.5 (27.2)	26.8 (28.4)	27.2 (27.4)	8.08 (2.80)

*Values in parentheses are for highest-resolution shell.

[a]Data were processed anisotropically via STARANISO included in the Autoproc suite.

[b]Resolution limits for three directions in reciprocal space (a*, b*, c*).

Table 2: **Refinement statistics**

	RhlR-P75 (mBTL)	RhlR-P75 (C4-HSL)	RhlR-P61 (mBTL)	PqsE-XTEN30-RhlR (mBTL)	PqsE/RhlR complex (mBTL)	PqsE-E187R
PDB entry	7R3G	7R3H	7R3I	7R3E	7R3J	7R3F
Resolution (Å)*	47.57-2.15 (2.22 - 2.15)	49.4-3.49 (3.62 - 3.49)	43.63-3.1 (3.21 - 3.1)	53.54-3.46 (3.71 - 3.46)	47.47 - 3.06 (3.17 - 3.064)	29.29 - 1.65 (1.71 - 1.65)
No. reflections	34603	12536	14454	19554 (ellipsoidal)	25592 (ellipsoidal)	43230
R_{work}*	0.199 (0.264)	0.223 (0.262)	0.248 (0.296)	0.228 (0.363)	0.269 (0.460)	0.206 (0.266)
R_{free}*	0.230 (0.307)	0.270 (0.347)	0.272 (0.346)	0.243 (0.405)	0.289 (0.475)	0.263 (0.291)
No. atoms						
Protein	3780	7278	3577	8610	8623	2339
Ligand/ion	72	50	72	76	76	21
Water	136	0	0	3	23	264
B-factors						
Protein	46.70	78.47	93.77	175.07	112.63	16.26
Ligand/ion	33.46	97.95	76.29	163.11	102.93	20.06
Water	44.32	0	0	178.51	90.71	24.49
R.m.s. deviations						
Bond lengths (Å)	0.003	0.002	0.002	0.003	0.003	0.005
Bond angles (°)	0.605	0.487	0.516	0.670	0.613	0.740
Ramachandran						
favored (%)	97.65	92.91	94.86	95.07	95.08	96.92
allowed (%)	1.92	6.23	4.71	4.47	4.27	3.08
outlier (%)	0.77	0.86	0.43	0.47	0.65	0.00

*Values in parentheses are for highest-resolution shell.