1 Supplemental Material

- 2 The supplemental material contains one supplementary table and six
- 3 supplementary figures.
- 4



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6 Figure S1. Protein-protein interfaces in RocR, related to Figure 1. A. The conserved EAL-EAL dimer interface. Superimposition of published EAL dimeric structures on the 7 EAL interface between subunits B and D of RocR. The two EAL dimers formed in RocR 8 (subunit B and D or their equivalent by the dyad subunits A and C) are structurally 9 similar to other EAL dimers known to be catalytically active. A similar dimerization 10 interface is seen, where Loop 6, α 8 and α 10 (helices shown as cylinders and loops as 11 tubes) are employed. The colour code is as follows: RocR. Cyan: EAL domain of subunit 12 B. Yellow: EAL domain of subunit D. Red sphere: Mg²⁺ in the EAL active site. TBD1265: 13 White. (PDB code, 2r6o, r.m.s.d. of 3.4 Å with the RocR EAL domain of subunit B); Ykul-14 EAL: Grev. (PDB code 2w27, r.m.s.d. 4.9 Å); BlrP1-EAL: Black. (PDB code 3gfz, r.m.s.d. 5.7 15 Å). **B.** The buried surface areas (BSA) as well as the number of hydrogen bonds (HB), salt 16 bridges (SB) are indicated for each interface that contributes to tetramer formation. 17



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Figure S2. RocR solution studies by SAXS, related to Figure 5. A. A hypothetical model 3 for a fully open RocR tetramer with 222 symmetry, related to Figure 5. All the REC 4 domains are exposed at the surface of the molecule. The four EAL active sites are 5 6 indicated by the presence of a c-di-GMP molecule, represented as a dumbbell. B. 7 Experimental scattering from the wild type protein (1) superimposed with the 8 scattering by the point mutants R286W (2) and D56N (3). The logarithm of the scattering intensity is plotted as a function of momentum transfer $s = 4 \pi \sin(\theta) / \lambda$, 9 10 where θ is the scattering angle and λ is the X-ray wavelength.



2 Figure S3. Structures of the REC, EAL domain and the inter-domain linker, related to Figure 3. **A**. The REC domain (subunit C) consists in a central β-sheet of five β-strands 3 (yellow arrows) surrounded by five α -helices (red tubes). The five residues from the 4 active site located at the ends of the β -strands (colored cyan) and the residue D56 5 (phosphorylation site, red) are labeled. E10, D10 and D56 coordinate the divalent metal 6 7 ion required for phosphorylation and dephosphorylation. S83 and K111 are proposed to 8 stabilize the phospho-aspartyl adduct and transmit signals to other parts of the protein. **B.** The EAL TIM barrel domain (subunit C) viewed from the top. Residues projecting 9 from the EVL motif and from Loop 6 (β 5- α 8) are shown as sticks (colored yellow and 10 pink). The Mg²⁺ ion is represented as a green sphere. **C.** View of the linker region 11 (residues 130-142) in subunit B with electron density displayed at a level of 1 σ 12 calculated using Fourier coefficients 2Fo-Fc and phases from the refined model. 13

RocR	REC	β1'	αl'	0000	β2'	α2'	β3'	0000
RocR BPA BAV CVI PSY VieA_VHA VieA_VVU BUR RPI	1 MNDL MKQF MGYLDKWKDR MHSL MKSL MRDL MRDL MRDL MRDL MRDL MRDL MCLGGVTHG MEGFAQL	I Q NVLVLED E DILVVEDH SVMVVDS KVLILEDH NVLIVEDE KILVVDH KILVVDH Metal bindir	29 PPQRLVAVTA PAHRLVVVHI PVQRLVLVRA ATHRLLAVGJ PFQLMALHQV PLQATNLKIJ PIHLTLMKQ(PVQSKVLQSN PVQRAAARQJ ggsite	ALKKVVPG ALRALGYTI LEEIGFG ULNANQVFI LNRLFEG QLAKIPNTI ATTKLGFD LHTLGVQ	SILEAADG RILEAADG RIIQAQEG TVHEAEHG DVLAADSV FITVVHRG RVATEQTV VCTAAS QILTACDG	40 KEAVATLES(NEALRRINE] SHALSQLEE LDAMNK(LAA) SEVATVCEA(ASALSTLSDI LNEARERLG REALYLLQL(50 CGHVDIAICD HGQVGIAICD GVADIVICD LDRVDLVVTD GGPVDIAICD D.HYDFVFCD TLAIDTVLLD C.HVDLVLCD Phosphoryl	6 0 LQMSGMDG IKMSGMDG LNMPYMDG LQMDGPDG IDLPDING LDMPHSDG LRLGNEAG IDMPAMNG attion site
RocR	α3' <u>000000000</u> 70	٩	β4'	<u>0000</u> 90	α4' 0000000 100	β5'	<u>000000</u> 120	x5' 0000000
RocR BPA BAV CVI PSY VieA_VHA VieA_VVU BUR RPI	LAFLRHASLS AQFLRVAARR TQFLREASQR VKLLESIAGK LELIRFLAES VNLLSTLAES IDLLISLNEG PELMEQMHLR	GL QL KL LQGK GQ SR KY AA GSRIFPRQ	UHSVILSSE LGAVIISSD VKSVILSSD VRFVAVMSG ARALIISA PKGVVILSA TGNVALISA PRSLILTSG APVWAWVSA	VDPILRQA VSSDLIAA VSSDLTAA VPRDVLDT SAACVLEG MTQDVLEI CDRPISA CDERTRTA MDAPIVES	FISMIECL VLDMAALI ILHMASLS VQGVVDAS VAQLALNQ TYNMCLSA VSAMCENF AVRLAHAY HVSLADAI	GLNFLGDLG GLRVLGDLA GLQVLGDLG ELNLLAVFP GLTVLGYLQ GYGFVRALT SFQVLGXLG GLTRTRGVQ	KPFSLERITA KPLDAQRLKA KPLKLSRLESK CPLKLAELSR CPASATALCE KPISNQQLTQ KPYSNNDIQQ KPYTLQQLSD KPLRPAHVLP	LLTRYNAR LLDRHEAE LLRRYESD VLDGYNPE LLKAYGPS IFGEFKQY LLDNAANA LLCTMPAC LLEAAAR
RocR	interdomain	linker	Е	α1 AL <u>2000</u>	0000 -	β1		32
RocR BPA BAV CVI PSY VieA_VHA VieA_VVU BUR RPI	RQDLF RQRARANAAF SAQPAGNKAT WSGTDESVSK RAELASPTPV LSQEQFVSLF LKPARKLRRR AWKPTPREVF ERDRAPAATA	PRQIE TASR SAP LSVS KNAPGALS VLMN VLNN RIDVS PRVTR GLPQ	140 VAEJ	LPSVADVVI APPAREVA SPEAIRAG KEDVAI GGPQDAAGJ DQEFLI ADVAJ SDHELAAL	5 0 RGLDNGEF RALAAGQI HALQQRQL ARSVAEQW FAFNEGRF FDLANGRV AALEGGHI IHETPEQI	160 EAYQOPKVA1 VPYFQPKVD1 IPYYQPKVS1 VPYYQPKVS1 INYYQPQYS1 XNYYQPLVD0 RPHFQPKVC1 EVVFQPQHD1	170 LDGGGLIGAE LLTLRPCGAE IKDDVLFGMB LQG.EVLGVB CRTGDVLGVB LASGGITGVB LASGGITGVB LASNQIAGAE *	180 VLARWNHP LARWNHP LLARWNHP LVRWCHP LVRWCHP ALVRCRHP ALARWLHP ALARWLHP ALARWSP ALCRWMHP
RocR	190	α2	210	α3	0000000	β3	α4 <u>00000</u> 0 240	α.5 0000000 250
RocR BPA BAV CVI PSY VieA_VHA VieA_VVU BUR RPI	HLGVLPPSHF DLGVLGPASF ILGLLPPSYF QYGVLSPAIF VHGLLAPGRF EHGIIAPAQF IHGMLSPAHF LFGNVPPDVF VHGRVSPAAF	LYVMETYN LEALKEQD IEAIKDCN VHHLEEGE MDTIEAEG ITEIQVN LPIVERCN IPVIEAEG VPRLEALG	LVDKLFWQL LLDRLIWHL BIDRLTLAL LAMRFFYQFI LTVALTWRV ELDKLFWIVI LSHELFDIV LSHELFDIV LADGLFFHVI	FSQGLATRI IDAALGQAI ADQAMAQTI LRDVCAAMI LDQALRLSJ LENALQDM IDNAIRDAI LRESLRACI LEHCVRVQI	RKLAQLG. RKLATAG. ARSALTG. KQFLALR. AQVLQAQG STLKVTI. RHINQGQ. AAWRHFF. HALSAHA.	Q PINLAFNYU ATQDLALMFI RGASLALMVI PGLHASINLI TALSVAVNI NLSVNMI RISINAI PDVSVAVNV; YPVSIGVNA;	HPSOLGSRAL ETSQLGSRAL ETCQLGNAQL PVPLLDDASL DPQVLQHADF NQKTLKQP.M DQINIEDGNF SP.ALVDQEL SAQTLSRAGT	AENISALL LPTLAQAL LNDLIAAL VDEMTSIV AEQIMQAL SERLFALC SERLFALC YDVIALL VDRIDAIV
RocR	η1 <u>000</u> 00-	β4 α	.6 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	α7 00000000	β5		x8	36 α9
RocR BPA CVI PSY VieA_VHA VieA_VVU BUR RPI	260 TEFHLPSIV RKHALPASIV ERYALPATAI KSSGLPSESI DRHDVPASAI DRHDVPASAI LENQVEPSVF HETHVPAESI REARIAPGAI	270 MFEITETG JTLEVTETG ALEVTETS TLEILEQC TLEILEQC TLEITENT JILEITENS AIELTEDS * *	288 LISAPASSLI LLDAPAATL LLSVQAATLI LLSVQAATLI LMSNLAASL VYDCSVTSLI SFSNSVALY ALGCSIQINI PVHDAAQLT	SILVRLRI ENLVRLRI STLVRLRI GTLARLRI GTLARLRI SALLRLCM ANLANLRI OVLTRLRI I ALNRLRI	290 MGCGLAMI MGCTVSII LGCTVSII NGFGLAMI QCKLSII RGVGLAII FGVQLSII LGHPLAII	300 DF GM GYSSL DF GT GFSSM DF GT GFSSM DF GT GFSSM DF GT GFSSM DF GT GFSSM DF GT GYSSL DF GT GYSSL DF GT GHSSL DF GT GHSSL DF GT GT ATL DF GT GT ATL DF GT GT ATL DF GT GT ATL	310 RICEFPFSQ RICHLPFNQ RICNLPFNQ COLSRSPFTE SQLATLPFSE SQLATLPFSE SQLATLPFTE SLFRLPFNE SLFRLPFNE	320 IKLDRTFV KIDASFV LKIDASFV LKIDREFV LKIDREFV LKIDRSFV LKIDRSFV LKLDRLFT LKIDRSFT
RocR	معمو معم	α10	β7		all	β8	200	x12 00000
RocR BPA CVI PSY VieA_VHA VieA_VVU BUR RPI	QKMKTQPRS RRLPGDARSE HDAASSPKKI RGMADDARKS KDALTNFKSQ SEMEIDSKKQ ANADRDQDAE AAVDQASQRG	AVISSVVA SVIAATLS SVIAATLS AILTSAVA AVVAGAMI QLTISSLH KIVNSICG RILRAVVA VICRTMIE	LAQALGISL MAERLGITVI LAESLGITVI MCQKLQLLSY MAQRLSMS LAKSLGLKSY LAKSLGLKSY LAKSLGLKSY LARSLGLKSY LARSLGLKSY	VVEGVESDI VAEGIENP JAEGIETAI VAEGVETRI JAEGVETRI JAEGVEDDI JAEGVEKQS JAEGVETE JAEGVETE	EQRVRLIE PQHQALLE EQWQQLAA DDFHSLLA ETLAYLRE STWNMLKK AVRHRLAR AQRQTLRA	LGCSTAQGYI LHCRQGQGYI MRCALGQGYI LGCDIAQGYI LGCDIAQGYI LGVELYQGFY YNIDVCQGYI MGCSLGQGWI LGCAIGQGYI	JRR PMPEQH JFAR PMSGPD VYAR PMSGDE YHSSPLPFDQ FIAR PMASA VRCR PIPFEQ JFNK PMPIEA LWSPAVAEEE LWAAPLSVTA	FLDYCSGS YARWLDGA YETWLFNP FMQWMRDS FLRWAAER LETLI INILSA LINVLNAA FLAHVQAA
RocR RocR								
BPA BAV CVI PSY VieA_VHA VieA_VVU BUR RPI	ASAAAG MVANT GDSRLQDGST GTRPRDPHEL A	ASGVQDMF	 LSCK 					

1 Figure S4. Sequence alignment of RocR homologs. Residues from the RocR sequence are numbered and the secondary structures elements are assigned based on the RocR 2 3 structure reported here. Sequence names are abbreviated as follows: BPA, Bordetella parapertussis (accession number: NP_883867); BAV, Bordetella avium 197N (accession 4 5 number: YP 786392); CVI, Chromobacterium violaceum ATCC 12472 (accession number: NP_902070); PSY, response regulator receiver in *Pseudomonas syringae* (accession 6 7 number: EGH64345); VieA VHA, response regulator VieA in Vibrio harvevi (accession number: YP_001448289); VieA_VVU, response regulator VieA in Vibrio vulnificus CMCP6 8 9 (accession number: NP_761989); BUR, response regulator receiver modulated diguanylate PDE in Burkholderia sp. Ch1-1 (accession number: 1ZP_06839714); RPI, 10 response regulator receiver modulated diguanylate PDE in Ralstonia pickettii 12J 11 (accession number: ZP 07675095). Strictly conserved residues are shaded in red and 12 boxed. Catalytically important regions are shaded in yellow; EAL residues essential for 13 PDE catalytic activity are marked with an asterisk. The roles of functionally important 14 residues are indicated below the sequences. Generated by using ESPript (Gouet et al., 15 1999). 16





Figure S5. Comparison of the spatial arrangement for BlrP1 and RocR, related to Figure

4 6. The black arrows indicate the pathways for the propagation of structural changes.



- **Figure S6**. Position of W286 at the REC-EAL interface, highlighted in this view rotated
- 4 around the y-axis by 180° compared to **Fig. 6**. W286 (green spheres) is adjacent to
- 5 residues Ile⁸⁹, Leu⁹⁰ of REC (blue spheres) and Phe³¹⁰, Pro³¹¹ of EAL (yellow spheres).

Supplementary Table 1. Protein-protein dimerization interfaces in the RocR tetramer, related to Figure 1.

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Interface	Dimer	N _{res}	A (Ų)	ΔG_{S} (kcal/mol)	N _{HB}	N _{SB}
А	C-D C, 42; D, 44		1630.5	-26.0	4	6
В	A-C	A, 38; D, 39	1357.4	-16.8	14	3
	B-D	B, 37; D, 40	1354.9	-16.5	18	3
	Average	1	1356.1	-16.7	16	3
С	B-C	B, 26; C, 28	825.0	-10.2	7	2
	A-D	A, 28; D, 29	880.2	-10.6	4	3
	Average	1	852.6	-10.4 6		3
D	A-B	A, 26; B, 27	952.6	-6.6	10	5

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6 N_{res} , number of residues buried; A, interface area; ΔG_S , solvation-energy change; N_{HB} ,

7 number of residues forming hydrogen bonds; N_{SB}, number of residues forming salt

8 bridges. All values are calculated using the Protein interfaces, surfaces and assemblies

9 service PISA at European Bioinformatics Institute

10 (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html).

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13 REFERENCES

14 Gouet, P., Courcelle, E., Stuart, D.I., and M \checkmark ©toz, F. (1999). ESPript: analysis of multiple sequence

alignments in PostScript. Bioinformatics 15, 305-308.

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