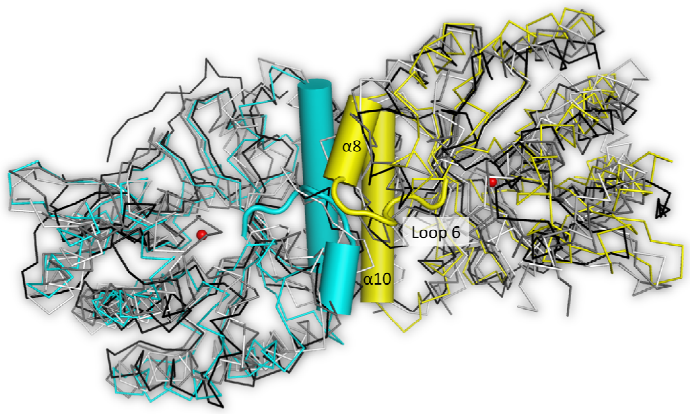
Supplemental Material

The supplemental material contains one supplementary table and six supplementary figures.

A



B

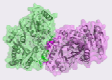
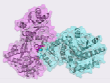
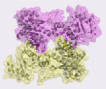
Interface		BSA (Å)	HB	SB
AB		952	10	5
AC, BD		1355	18	3
AD, BC		825	7	2
CD		1631	4	6

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 EAL interface between subunits B and D of RocR. The two EAL dimers formed in RocR (subunit B and D or their equivalent by the dyad subunits A and C) are structurally similar to other EAL dimers known to be catalytically active. A similar dimerization interface is seen, where Loop 6, α8 and α10 (helices shown as cylinders and loops as tubes) are employed. The colour code is as follows: RocR. Cyan: EAL domain of subunit B. Yellow: EAL domain of subunit D. Red sphere: Mg^{2+} in the EAL active site. TBD1265: White. (PDB code, 2r6o, r.m.s.d. of 3.4 Å with the RocR EAL domain of subunit B); YkuI-EAL: Grey. (PDB code 2w27, r.m.s.d. 4.9 Å); BlrP1-EAL: Black. (PDB code 3gfz, r.m.s.d. 5.7 Å). **B.** The buried surface areas (BSA) as well as the number of hydrogen bonds (HB), salt bridges (SB) are indicated for each interface that contributes to tetramer formation.

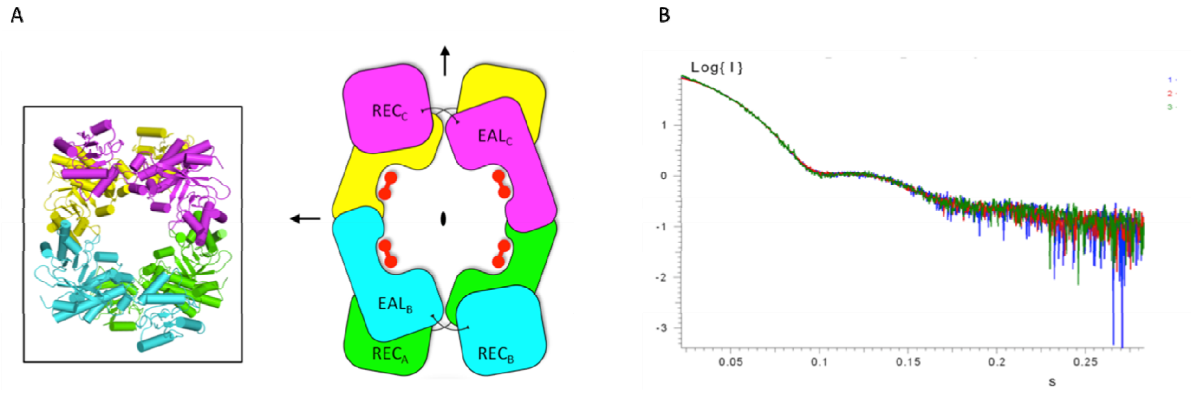


Figure S2. RocR solution studies by SAXS, related to Figure 5. **A.** A hypothetical model for a fully open RocR tetramer with 222 symmetry, related to Figure 5. All the REC domains are exposed at the surface of the molecule. The four EAL active sites are indicated by the presence of a c-di-GMP molecule,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

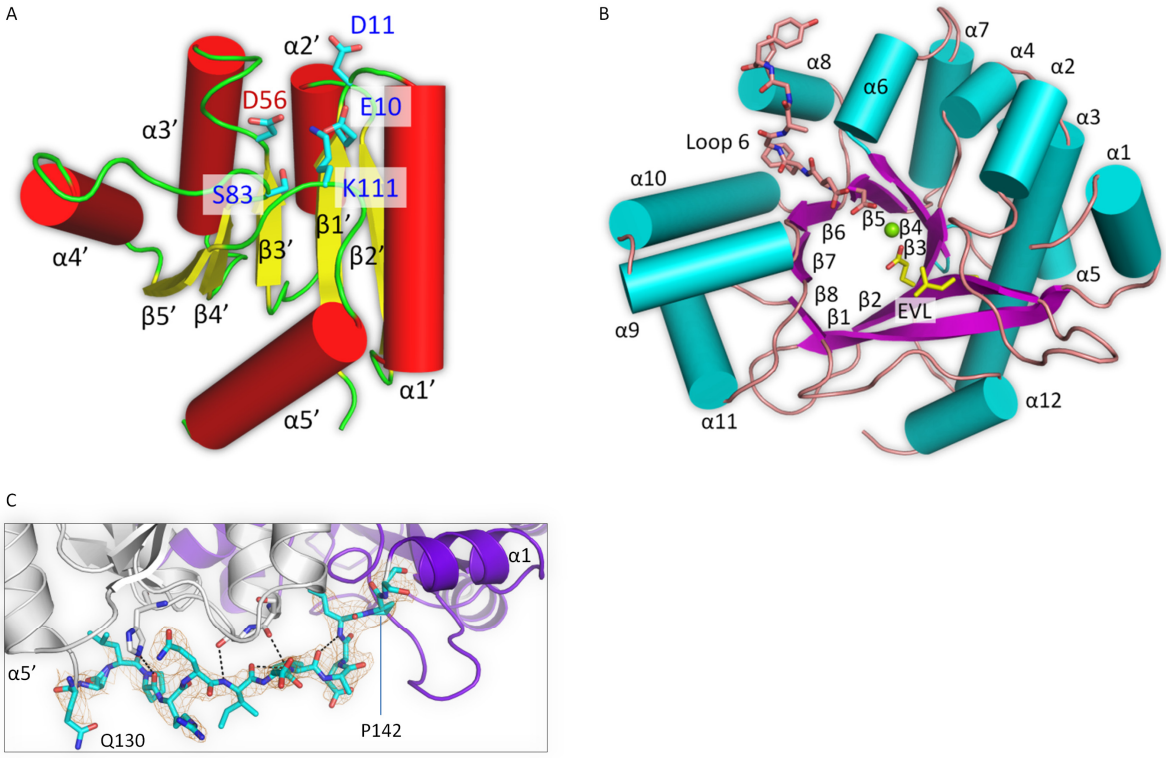


Figure S3. Structures of the REC, EAL domain and the inter-domain linker, related to Figure 3. **A**. The REC domain (subunit C) consists in a central β-sheet of five β-strands (yellow arrows) surrounded by five α-helices (red tubes). The five residues from the active site located at the ends of the β-strands (colored cyan) and the residue D56 (phosphorylation site, red) are labeled. E10, D10 and D56 coordinate the divalent metal ion required for phosphorylation and dephosphorylation. S83 and K111 are proposed to 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 from the EVL motif and from Loop 6 (β5-α8) are shown as sticks (colored yellow and pink). The Mg^{2+} ion is represented as a green sphere. **C.** View of the linker region (residues 130-142) in subunit B with electron density displayed at a level of 1 σ calculated using Fourier coefficients 2Fo-Fc and phases from the refined model.

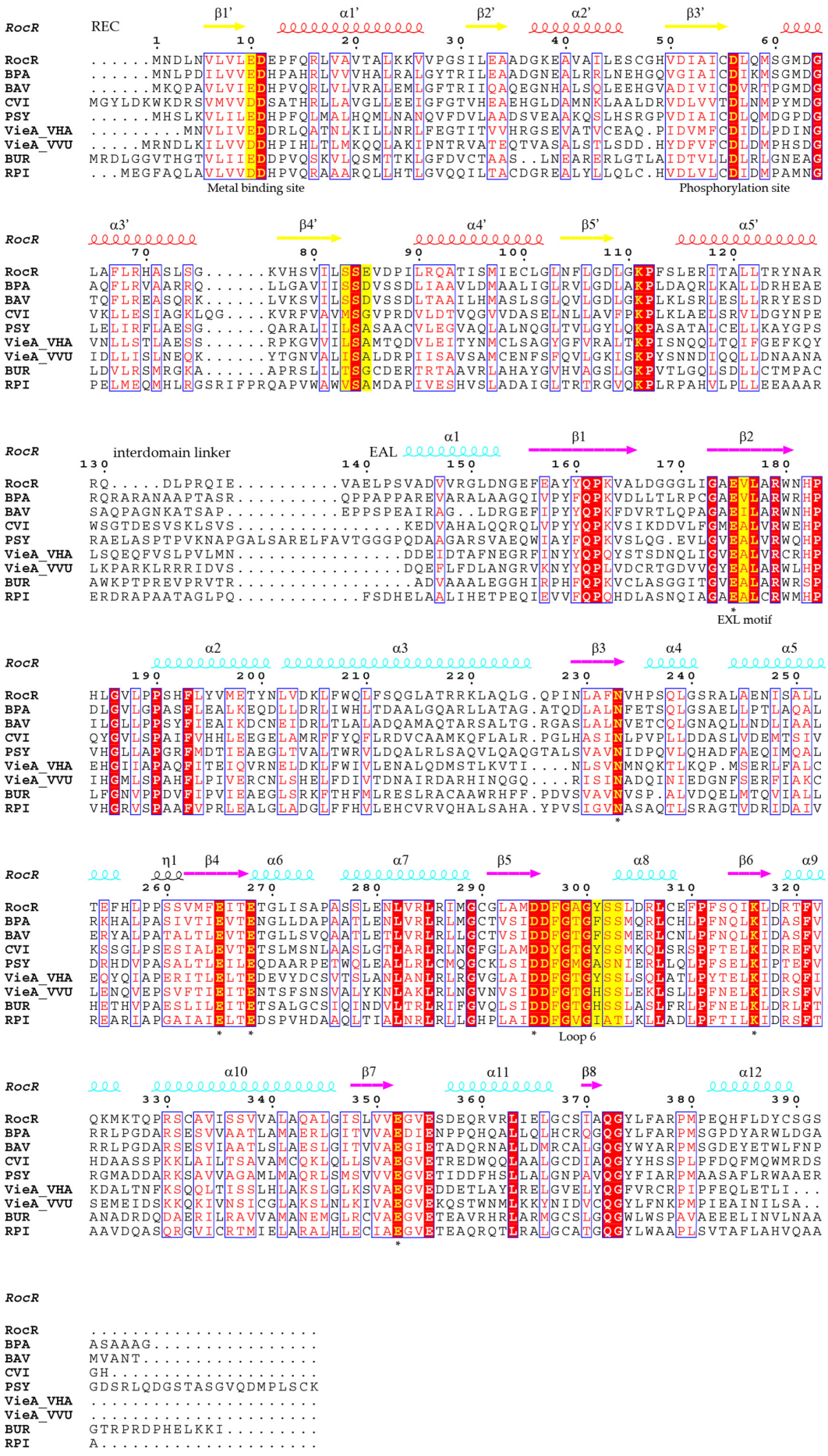

RocR
REC
β1' α1' β2' α2' β3'
Metal binding site
Phosphorylation site
α3' β4' α4' β5' α5'
interdomain linker
EAL
α1 β1 β2
EXL motif
α2 α3 β3 α4 α5
η1 β4 α6 α7 β5 α8 β6 α9
Loop 6
α10 β7 α11 β8 α12
RocR
BPA
BAV
CVI
PSY
VieA_VHA
VieA_VVU
BUR
RPI

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 structure reported here. Sequence names are abbreviated as follows: BPA, *Bordetella parapertussis* (accession number: NP_883867); BAV, *Bordetella avium* 197N (accession number: YP_786392); CVI, *Chromobacterium violaceum* ATCC 12472 (accession number: NP_902070); PSY, response regulator receiver in *Pseudomonas syringae* (accession number: EGH64345); VieA_VHA, response regulator VieA in *Vibrio harveyi* (accession number: YP_001448289); VieA_VVU, response regulator VieA in *Vibrio vulnificus* CMCP6 (accession number: NP_761989); BUR, response regulator receiver modulated diguanylate PDE in *Burkholderia* sp. Ch1-1 (accession number: 1ZP_06839714); RPI, response regulator receiver modulated diguanylate PDE in *Ralstonia pickettii* 12J (accession number: ZP_07675095). Strictly conserved residues are shaded in red and boxed. Catalytically important regions are shaded in yellow; EAL residues essential for PDE catalytic activity are marked with an asterisk. The roles of functionally important residues are indicated below the sequences. Generated by using ESPript (Gouet et al., 1999).

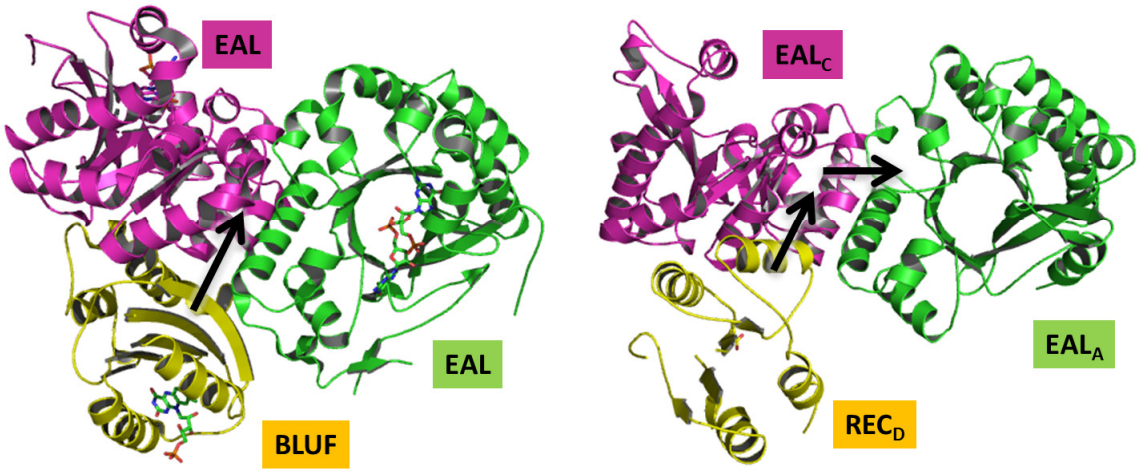


Figure S5. Comparison of the spatial arrangement for BlrP1 and RocR, related to Figure 6. The black arrows indicate the pathways for the propagation of structural changes.

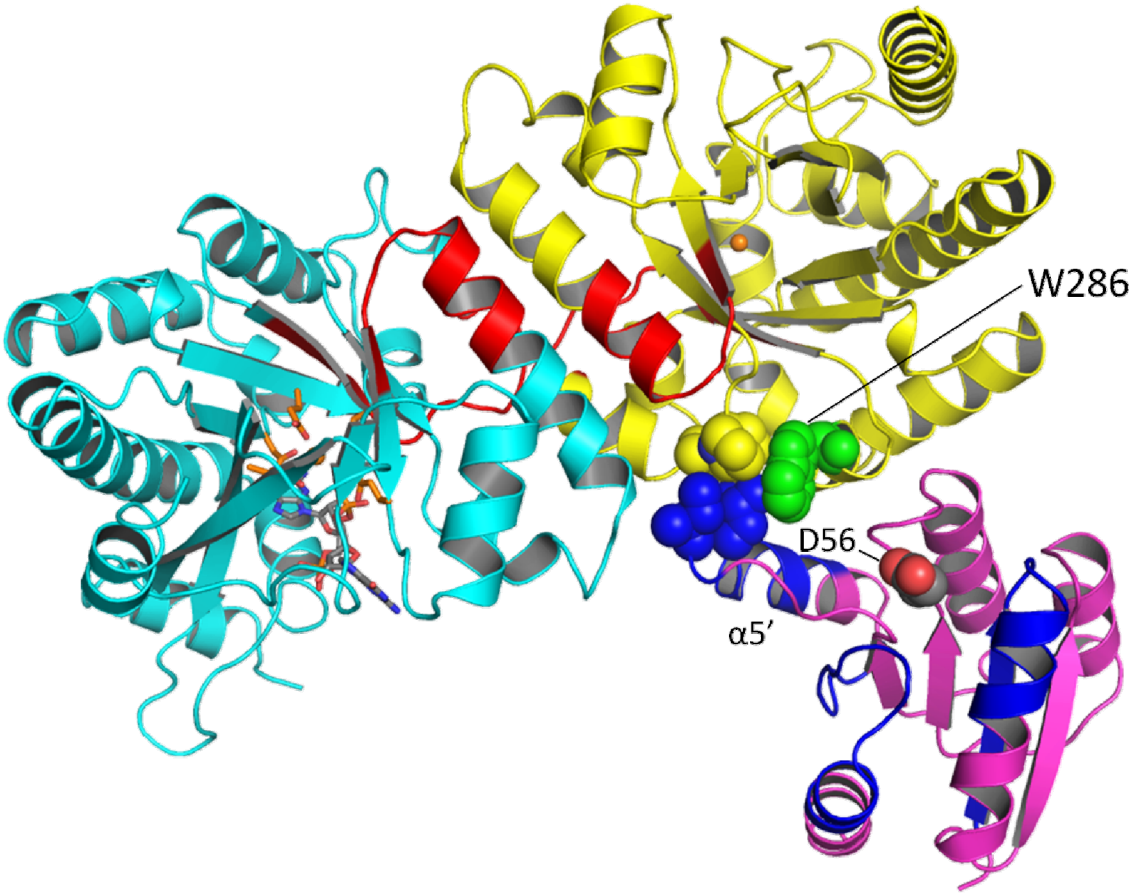


Figure S6. Position of W286 at the REC-EAL interface, highlighted in this view rotated around the y-axis by 180° compared to **Fig. 6**. W286 (green spheres) is adjacent to residues Ile^{89}, Leu^{90} of REC (blue spheres) and Phe^{310}, Pro^{311} of EAL (yellow spheres).

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

Interface	Dimer	N_{res}	A (Å^2)	ΔG_S (kcal/mol)	N_{HB}	N_{SB}
A	C-D	C, 42; D, 44	1630.5	-26.0	4	6
B	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		1356.1	-16.7	16	3
C	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		852.6	-10.4	6	3
D	A-B	A, 26; B, 27	952.6	-6.6	10	5

N_{res}, number of residues buried; A, interface area; ΔG_S, solvation-energy change; N_{HB}, number of residues forming hydrogen bonds; N_{SB}, number of residues forming salt bridges. All values are calculated using the Protein interfaces, surfaces and assemblies service PISA at European Bioinformatics Institute (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html).

REFERENCES

Gouet, P., Courcelle, E., Stuart, D.I., and M √ ©toz, F. (1999). ESPript: analysis of multiple sequence alignments in PostScript. Bioinformatics *15*, 305-308.