

Supplementary Information

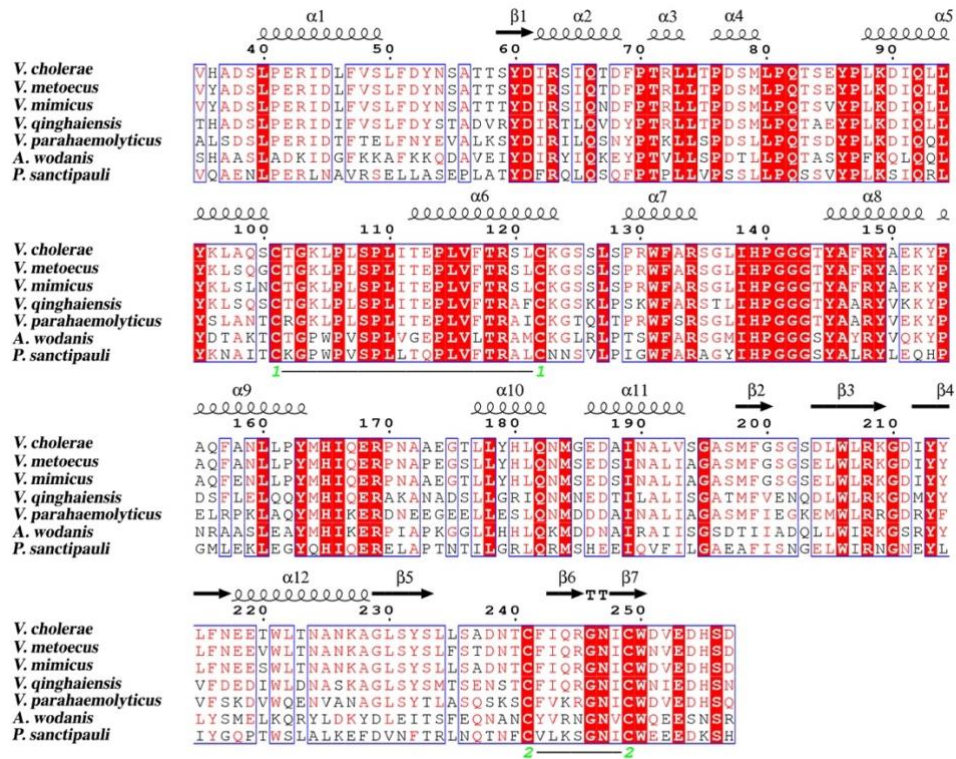


Figure S1. Structure-based multiple sequence alignment of VxrA-SD and its homologs.

The sequences of SDs of *Vibrio cholerae* (NP_232955.1), *Vibrio metoecus* (KQA20136.1), *Vibrio mimicus* (EEY46071.1), *Vibrio qinghaiensis* (WP_094501440.1), *Vibrio parahaemolyticus* (WP_025533427.1), *Aliivibrio wodanis* (WP_045104543.1) and *Photobacterium sanctipauli* (WP_036817922.1) were used in the alignment. The α -helices and β -strands are marked with coils and arrows, respectively, above the appropriate sequences of *Vibrio cholerae* VxrA-SD based on its structure. The alignment was generated with the programs MultAlin (1) and ESPrpt (2) and was modified slightly for clarity.

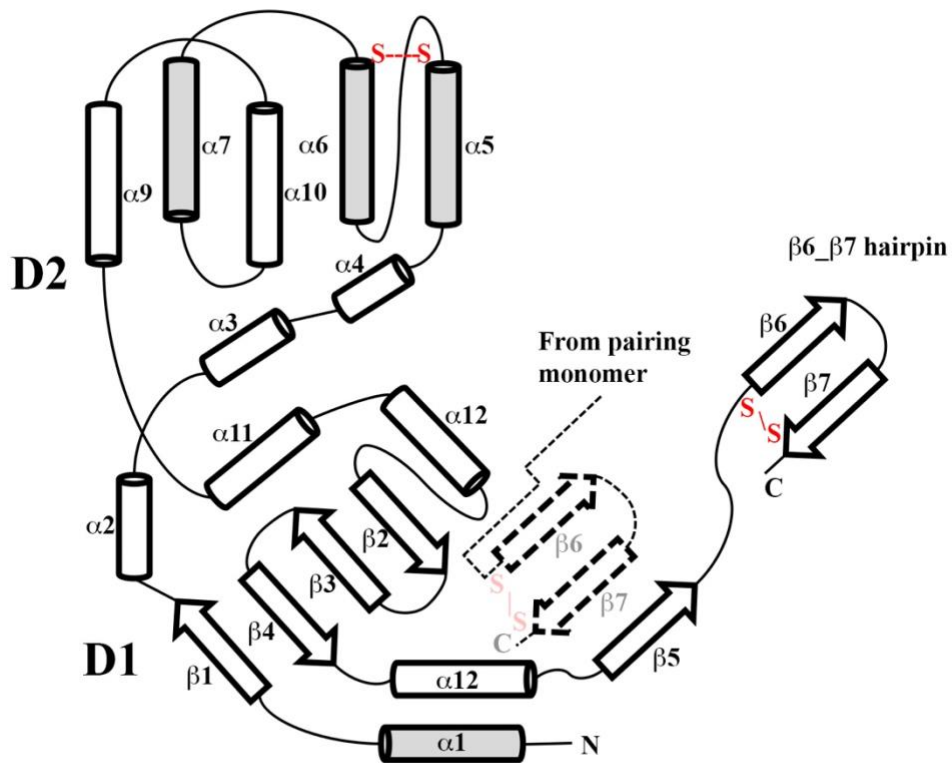


Figure S2. The scheme of VxrA-SD structure. In the diagram, β strands are represented by arrows and α helices are represented by cylinders. The red S-S labels show the locations of disulfide bonds.

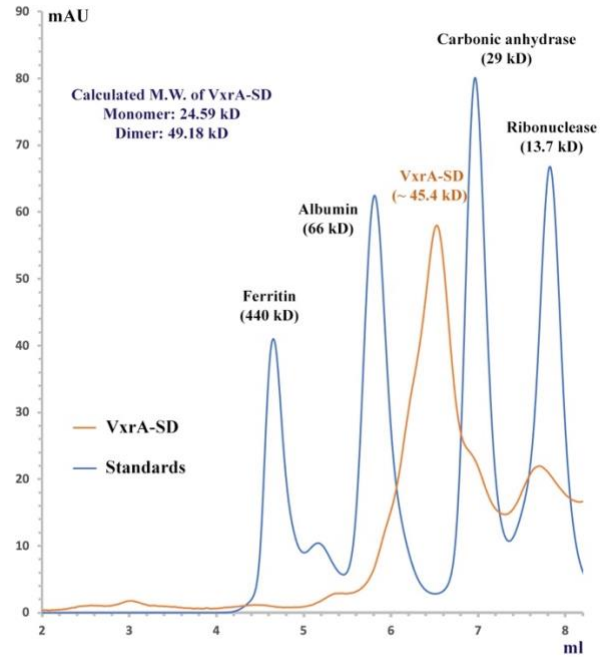


Figure S3. Size exclusion chromatography of VxrA-SD. Predominant species of VxrA-SD migrates on the gel with the apparent molecular mass of about 45.4 kD, which is consistent with that of a VxrA-SD dimer. mAU is milli-absorbance unit.

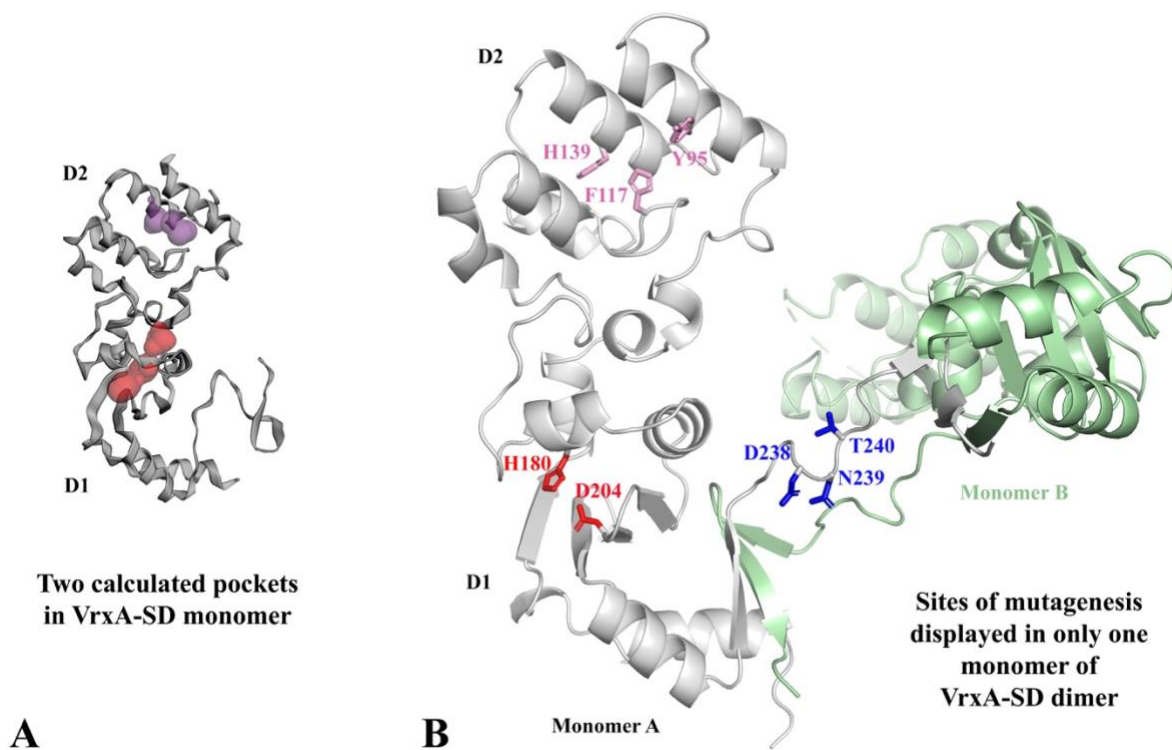


Figure S4. Potential binding pockets of signaling molecule and targets of mutagenesis. (A) Two major pockets of VrxA-SD based on the calculation with the program CASTp (3). (B) Sites of mutagenesis. The sites of some prominent pocket-forming residues selected for mutagenesis study are colored in pink or red. Those residues targeted in β -linker-shortening mutagenesis are colored in blue. All targeted residues are shown in stick format in only one monomer of VrxA-SD dimer.

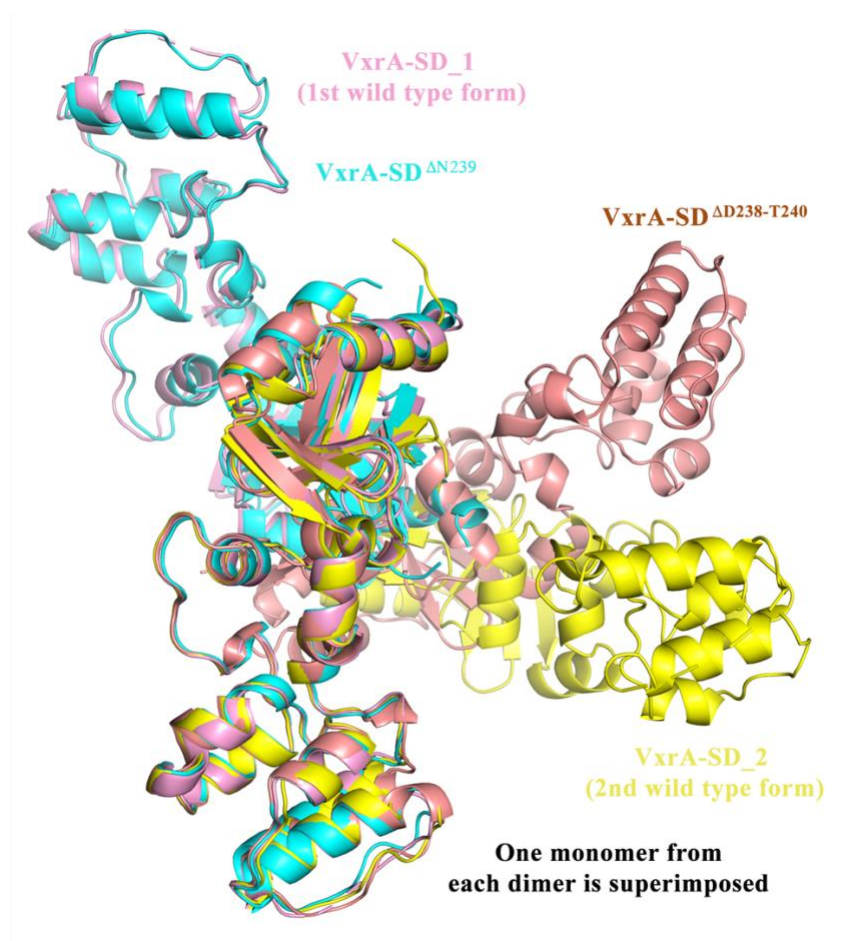


Figure S5. Conformational changes of VxrA-SD dimer. The dimers of two wild types, VxrA-SD_1 and VxrA-SD_2, and the dimers of two mutants, VxrA-SD^{ΔN239} and VxrA-SD^{ΔD238-T240} are superimposed with one monomer of each dimer. For clarity, VxrA-SD^{ΔN239-T240} dimer that is nearly identical to VxrA-SD^{ΔN239} dimer in conformation is not included in the alignment. The relative positions (rotation) of their second monomer illustrate the conformational changes of VxrA-SD dimer. The wild type VxrA-SD_2 has the largest relative conformational change in comparison to VxrA-SD_1 while VxrA-SD^{ΔD238-T240} shows an intermedium conformational change.

Table S1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Relevant genotype	Source
<i>E. coli</i> strains		
CC118 λ pir	$\Delta(\text{ara-leu})$ <i>araD</i> ΔlacX74 <i>galE galK phoA20 thi-1 rpsE rpoB argE(Am) recA1</i> λ pir	(4)
S17-1 λ pir	Tp ^f Sm ^r 6ec <i>Athi pro</i> r _K ⁻ m _K ⁺ RP4::2-Tc::MuKm Tn7 λ pir	(5)
<i>V. cholerae</i> strains		
FY_VC_9332	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA	(6)
FY_VC_14821	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA , Tn7:: <i>vxrA</i> ^{MH} , Rif ^r	This study
FY_VC_14823	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA , Tn7:: <i>vxrA</i> ^{MH, C241AC249A} , Rif ^r	This study
FY_VC_14825	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA , Tn7:: <i>vxrA</i> ^{MH, C101AC122A} , Rif ^r	This study
FY_VC_14824	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA , Tn7:: <i>vxrA</i> ^{MH, ΔN239-T240} , Rif ^r	This study
FY_VC_14826	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA , Tn7:: <i>vxrA</i> ^{MH, ΔD238-T240} , Rif ^r	This study
FY_VC_14827	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA , Tn7:: <i>vxrA</i> ^{MH, ΔN239} , Rif ^r	This study
FY_VC_14828	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA , Tn7:: <i>vxrA</i> ^{MH, Y95A} , Rif ^r	This study
FY_VC_14830	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA , Tn7:: <i>vxrA</i> ^{MH, F117A} , Rif ^r	This study
FY_VC_14832	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA , Tn7:: <i>vxrA</i> ^{MH, H180A} , Rif ^r	This study
FY_VC_14831	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA , Tn7:: <i>vxrA</i> ^{MH, D204A} , Rif ^r	This study
FY_VC_14829	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA , Tn7:: <i>vxrA</i> ^{MH, H139A} , Rif ^r	This study
FY_VC 14470	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA $\Delta\text{lacZ-vpsLp}::\text{lacZ}$ pBAD Rif ^r	This study
FY_VC 15724	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA $\Delta\text{lacZ-vpsLp}::\text{lacZ}$ pBAD- <i>vxrA</i> ^{MH} , Rif ^r	This study
FY_VC 15726	<i>Vibrio cholerae</i> O1 El Tor A1552, ΔvxrA $\Delta\text{lacZ-vpsLp}::\text{lacZ}$ pBAD- <i>vxrA</i> ^{MH, C241A/C249A} , Rif ^r	This study

FY_VC 15725	<i>Vibrio cholerae</i> O1 El Tor A1552, $\Delta vxrA$ $\Delta lacZ-vpsLp::lacZ$ pBAD- <i>vxrA</i> ^{MH} , C101A/C122A, Rif ^r	This study
FY_VC 15728	<i>Vibrio cholerae</i> O1 El Tor A1552, $\Delta vxrA$ $\Delta lacZ-vpsLp::lacZ$ pBAD- <i>vxrA</i> ^{MH, $\Delta N239-T240$} , Rif ^r	This study
FY_VC 15729	<i>Vibrio cholerae</i> O1 El Tor A1552, $\Delta vxrA$ $\Delta lacZ-vpsLp::lacZ$ pBAD- <i>vxrA</i> ^{MH, $\Delta D238-T240$} , Rif ^r	This study
FY_VC 15727	<i>Vibrio cholerae</i> O1 El Tor A1552, $\Delta vxrA$ $\Delta lacZ-vpsLp::lacZ$ pBAD- <i>vxrA</i> ^{MH, $\Delta N239$} , Rif ^r	This study
FY_VC 15730	<i>Vibrio cholerae</i> O1 El Tor A1552, $\Delta vxrA$ $\Delta lacZ-vpsLp::lacZ$ pBAD- <i>vxrA</i> ^{MH, Y95A} , Rif ^r	This study
FY_VC 15731	<i>Vibrio cholerae</i> O1 El Tor A1552, $\Delta vxrA$ $\Delta lacZ-vpsLp::lacZ$ pBAD- <i>vxrA</i> ^{MH, F117A} , Rif ^r	This study
FY_VC 15733	<i>Vibrio cholerae</i> O1 El Tor A1552, $\Delta vxrA$ $\Delta lacZ-vpsLp::lacZ$ pBAD- <i>vxrA</i> ^{MH, H180A} , Rif ^r	This study
FY_VC 15734	<i>Vibrio cholerae</i> O1 El Tor A1552, $\Delta vxrA$ $\Delta lacZ-vpsLp::lacZ$ pBAD- <i>vxrA</i> ^{MH, D204A} , Rif ^r	This study
FY_VC 15732	<i>Vibrio cholerae</i> O1 El Tor A1552, $\Delta vxrA$ $\Delta lacZ-vpsLp::lacZ$ pBAD- <i>vxrA</i> ^{MH, H139A} , Rif ^r	This study
Plasmids		
pUX-BF13	oriR6K helper plasmid, <i>mob/oriT</i> , provides the Tn7 transposition function in <i>trans</i> , Ap ^r	(7)
pMCM11	pGP704::mTn7- <i>gfp</i> , Gm ^r Ap ^r	M. Miller and G. Schoolnik
pFY-6334	pGP704-Tn7- <i>vxrA</i> ^{MH} , Gm ^r , Ap ^r	This study
pFY-6335	pGP704-Tn7- <i>vxrA</i> ^{MH, C241A/C249A} , Gm ^r , Ap ^r .	This study
pFY-6337	pGP704-Tn7- <i>vxrA</i> ^{MH, C101A/C122A} , Gm ^r , Ap ^r .	This study
pFY-6336	pGP704-Tn7- <i>vxrA</i> ^{MH, $\Delta N239-T240$} , Gm ^r , Ap ^r .	This study
pFY-6338	pGP704-Tn7- <i>vxrA</i> ^{MH, $\Delta D238-T240$} , Gm ^r , Ap ^r .	This study
pFY-6339	pGP704-Tn7- <i>vxrA</i> ^{MH, $\Delta N239$} , Gm ^r , Ap ^r .	This study
pFY-6340	pGP704-Tn7- <i>vxrA</i> ^{MH, Y95A} , Gm ^r , Ap ^r .	This study
pFY-6342	pGP704-Tn7- <i>vxrA</i> ^{MH, F117A} , Gm ^r , Ap ^r .	This study
pFY-6344	pGP704-Tn7- <i>vxrA</i> ^{MH, H180A} , Gm ^r , Ap ^r .	This study
pFY-6343	pGP704-Tn7- <i>vxrA</i> ^{MH, D204A} , Gm ^r , Ap ^r .	This study

pFY-6341		pGP704-Tn7- <i>vxrA</i> ^{MH, H139A} , Gm ^r , Ap ^r ,	This study
pBAD/myc B	His-	Arabinose-inducible expression vector with C-terminal myc epitope and six-His tags	
pFY-6417		pBAD- <i>vxrA</i> ^{MH} , Ap ^r	This study
pFY-6419		pBAD- <i>vxrA</i> ^{MH, C241A/C249A} , Ap ^r ,	This study
pFY-6418		pBAD- <i>vxrA</i> ^{MH, C101A/C122A} , Ap ^r ,	This study
pFY-6421		pBAD- <i>vxrA</i> ^{MH, ΔN239-T240} , Ap ^r ,	This study
pFY-6422		pBAD- <i>vxrA</i> ^{MH, ΔD238-T240} , Ap ^r ,	This study
pFY-6420		pBAD- <i>vxrA</i> ^{MH, ΔN239} , Ap ^r ,	This study
pFY-6423		pBAD- <i>vxrA</i> ^{MH, Y95A} , Ap ^r ,	This study
pFY-6424		pBAD- <i>vxrA</i> ^{MH, F117A} , Ap ^r ,	This study
pFY-6426		pBAD- <i>vxrA</i> ^{MH, H180A} , Ap ^r ,	This study
pFY-6427		pBAD- <i>vxrA</i> ^{MH, D204A} , Ap ^r ,	This study
pFY-6425		pBAD- <i>vxrA</i> ^{MH, H139A} , Ap ^r ,	This study

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