Supporting Information

Structural insights into the molecular mechanism of *Escherichia coli* SdiA, a quorum sensing receptor

Truc Kim,[a] Thao Duong,[a] Chun-ai Wu,[a] Jongkeun Choi,[b] Nguyen Lan,[a] Sung Wook Kang,[a] Neratur K. Lokanath,[c] Dong-Woo Shin,[a] Hye-Yeon Hwang,[a] and Kyeong Kyu Kim[a]

[a]Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon 440-746, Korea

[b]Department of Cosmetic Science, Chungwoon University, San 29, Namjang, Hongsung, Chungnam 350-701, Korea

[c]Department of Studies in Physics, University of Mysore, Manasagangotri, Mysore 570 006, India

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Supporting Materials and Methods

Size-exclusion chromatography

For size exclusion chromatography analysis, 100 μl of protein solution containing approximately 0.1 mM SdiA in an apo- or holo-form was injected into a Superdex 75 10/300 GL column (GE Healthcare, Sweden) connected to an ÄKTA FPLC system (GE Healthcare, Sweden) with a UV280 detector. To prepare the holo-form of SdiA, protein was mixed with C_8-HSL prepared in DMSO at a molar ratio of one protein molecule to three AHLs in buffer containing 25 mM HEPES pH 7.5, 1 M NaCl, and 5 mM DTT, and incubated on ice for 1 h. Proteins were eluted by the same buffer at a flow rate of 0.5 ml min^{-1}, and the elution volume versus absorbance at 280 nm was monitored. A gel filtration low-molecular-weight calibration kit (GE Healthcare, Sweden) was used to calibrate the gel filtration column and estimate the size of SdiA.

Supporting Figures and Figure Legends

Figure S1

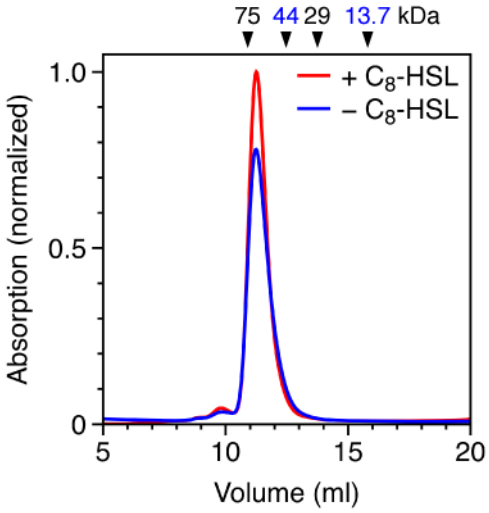

Fig. S1. Size-exclusion chromatography profile of SdiA in the presence (*red*) or absence (*blue*) of C_8-HSL. Molecular weight markers are shown at the top: conalbumin (75 kDa), ovalbumin (44 kDa), carbonic anhydrase (29 kDa), and ribonuclease A (13.7 kDa).

Figure S2

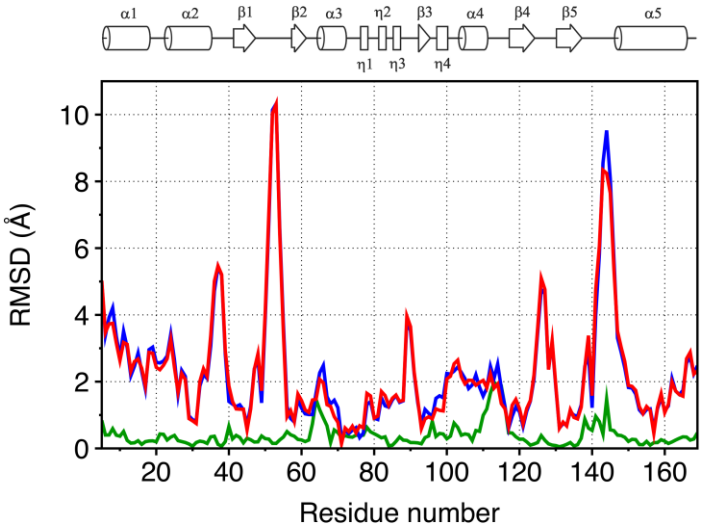

Fig. S2. Rmsd per residue values of the N-terminal LBDs of SdiA (residues 5-169): C2 versus $P6_522$ crystal (*green*), C2 crystal structure versus the mean solution structure (*blue*), and $P6_522$ crystal structure versus the mean solution structure (*red*). Secondary structure elements of the SdiA crystal structure are shown at the top.

Figure S3

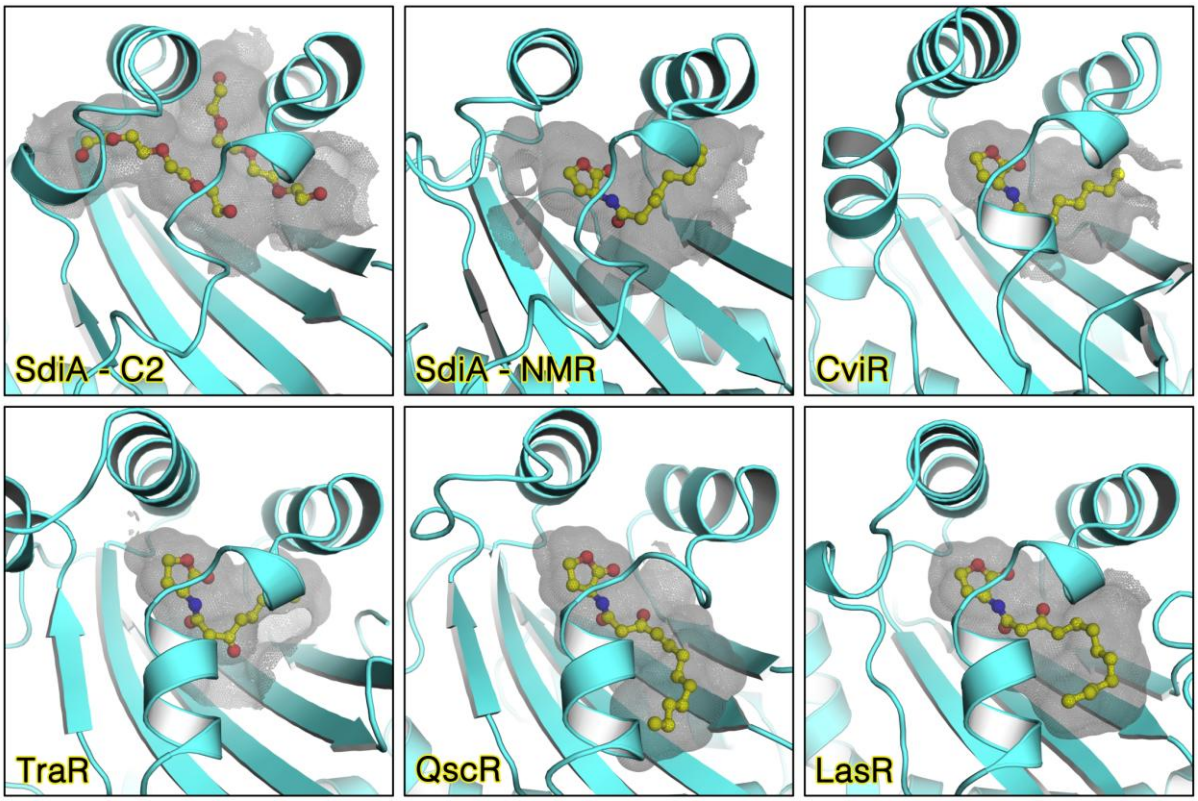

Fig. S3. Ligand-binding pockets of the LuxR-type receptors. Binding pockets are shown as gray mesh. Bound ligands are illustrated as yellow ball-and-stick models. LBDs are shown as ribbon diagrams.

Figure S4

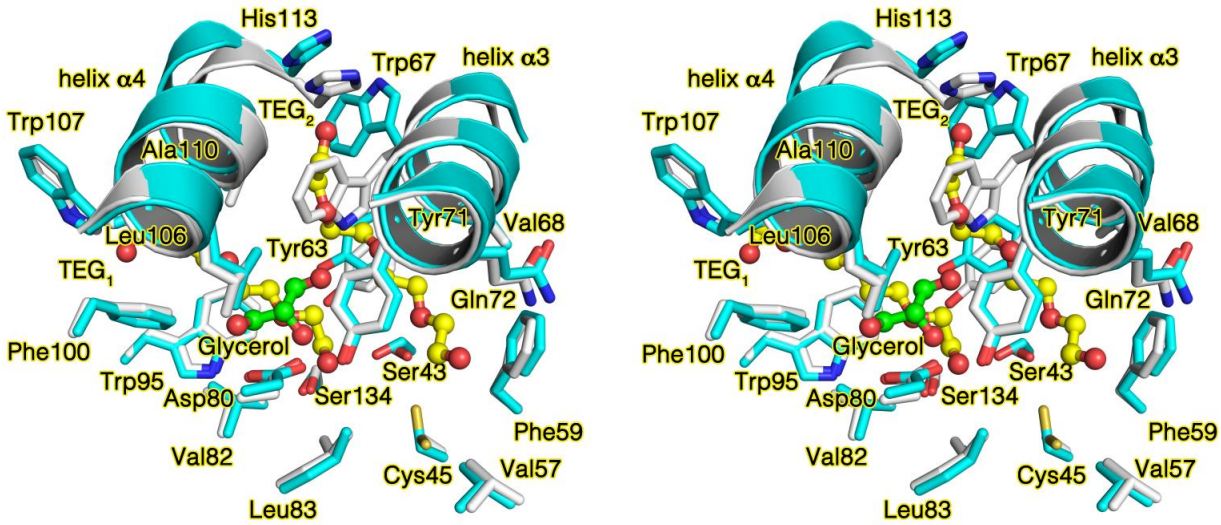

Fig. S4. Stereo view of structural superimposition of the ligand-binding sites of SdiA in the C2 (*cyan*) and $P6_522$ (*white*) crystal structures. Helices α3 and α4 are shown as ribbon diagrams, and residues in the ligand pockets are shown as sticks. Tetraethylene glycol (TEG) (*yellow*) and glycerol (*green*) molecules are illustrated as ball-and-stick models.

Figure S5

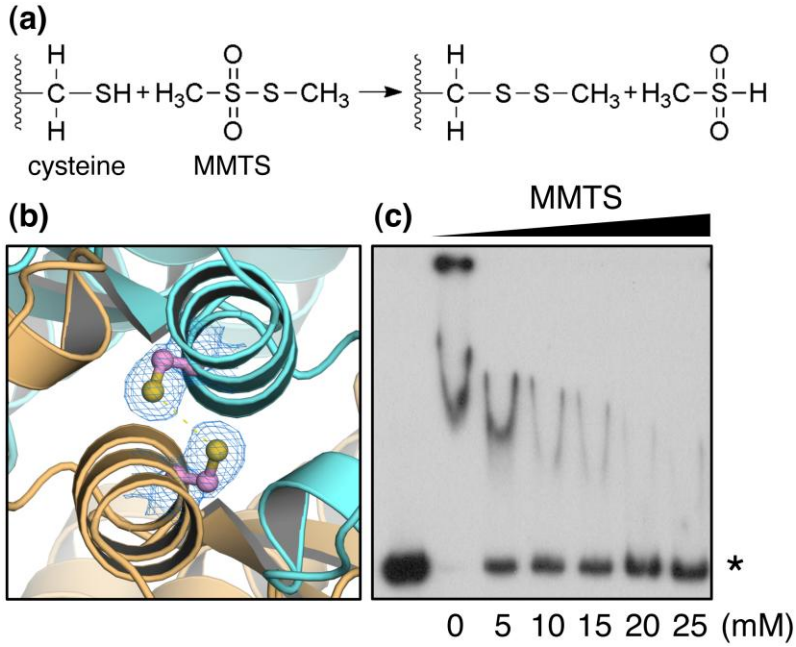

Fig. S5. The MMTS-modified functional dimer form of SdiA C-terminal DBD. (a) Modification of cysteine residues by MMTS. (b) Position of the Cys232-Cys232 bond in the ribbon diagram of an SdiA dimer. Figure illustration is as in Figure 6a. (c) Gel-shift assay of *uvrY* promoter in the presence of MMTS-modified SdiA. Protein was mixed with various concentrations of methyl methane thiosulfonate (MMTS) prior to the assay. The first lane contains the DNA probe only. The concentration of MMTS is indicated in each lane, and the star indicates free *uvrY* promoter.

Supporting Tables

Table S1. Residues involved in monomer subunit interaction of dimeric SdiA

Interactions	Subunit 1		Subunit 2	
	Location	**Residue**	**Location**	**Residue**
Salt bridge	helix α1	Arg11	helix α5	Asp148
Hydrogen bond	loop β1-β2	Phe52	helix α6	Glu193
	loop β1-β2	Thr53	helix α6	Glu193
	loop α3-β3	Gln90	helix α5	Glu160
	strand β4	Met124	strand β5	Arg128
	helix α6	Glu193	helix α9	Tyr233
	helix α9	Thr228	helix α9	Thr228
	helix α9	Cys232	helix α9	Cys232
van der Waals	helix α1	Phe6	helix α5	Leu152
	helix α1	Phe7	helix α5	Asp148
	helix α1	Phe7	loop β5-α5	Leu146
	helix α1	Phe7	helix α5	Leu152
	helix α1	Phe7	helix α5	Glu149
	helix α1	Arg10	helix α5	Gln151
	helix α1	Arg11	helix α5	Asp148
	loop β1-β2	Pro51	helix α6	Glu193
	loop β1-β2	Phe52	helix α6	Glu193
	loop β1-β2	Phe52	helix α6	Arg189
	loop β1-β2	Phe52	C-end	Ile240
	loop β1-β2	Phe52	helix α6	Ala192
	loop β1-β2	Thr53	helix α6	Glu193
	loop β1-β2	Thr53	helix α6	Trp190
	loop α3-β3	Glu90	helix α5	Arg159
	strand β4	Met124	strand β5	Arg128
	strand β4	Met124	strand β4	Met124
	strand β4	Met124	strand β4	Leu125
	strand β5	Arg128	strand β5	Arg128
	helix α6	Thr191	helix α9	Thr228
	helix α6	Ala192	helix α9	Cys232
	helix α6	Ala192	helix α9	Gln229
	helix α6	Glu193	helix α9	Gln229
	helix α6	Glu193	helix α9	Tyr233
	loop α6-α7	Gly194	loop α8-α9	Asn226
	helix α9	Thr228	helix α9	Thr228
	helix α9	Ala231	helix α9	Cys232
	helix α9	Cys232	helix α9	Ala235
	helix α9	Ala235	helix α9	Ala236

Table S2. Structural alignment of SdiA to its homologues

	LBD[a]		DBD[b]		Full	
	residues	**rmsd (Å)/Cα**	**residues**	**rmsd (Å)/Cα**	**residues**	**rmsd (Å)/Cα**
TraR[c]	2-165	3.48/152	176-234	0.84/56	2-234	3.84/160
CviR[d]	18-186	3.55/152	200-258	1.65/56	18-258	4.71/176
CviR[e]	18-186	3.46/152	200-258	1.44/56	18-258	5.53/184
CviR[f]	18-186	3.46/152				
QscR	5-168	2.44/152	178-236	0.87/56	5-236	2.86/224
LasR	6-168	2.88/152				

[a] SdiA LBD (residues 5-168)
[b] SdiA DBD (residues 182-240)
[c] TraR chain A (PDB: 1L3L)
[d] CviR structure in complex with antagonist chlorolactone (PDB: 3QP5)
[e] CviR structure of *C. violaceum* 12472 in complex with C_6-HSL (PDB: 3QP6)
[f] CviR structure in complex with C_8-HSL (PDB: 3QP2)

Table S3. Primers used in this study

Primer name	Primer sequence (5'-3')	Purpose
*sdiA*C45S-F	CGATTACTATTCGTTATCTGTCCGCCACCCGGTACC	mutagenesis of Cys45 to Ser45 in *E. coli* SdiA
*sdiA*C45S-R	GGTACCGGGTGGCGGACAGATAACGAATAGTAATCG	
*sdiA*C138S-F	CTTTTTGTCCTTTTCCCGTTCCAGCGCGCGCGAAATACCC	mutagenesis of Cys138 to Ser138 in *E. coli* SdiA
*sdiA*C138S-R	GGGTATTTCGCGCGCGCTGGAACGGGAAAAGGACAAAAAG	
*sdiA*C232S-F	CCCAGGTTGCCTCTTACGCGGCCGC	mutagenesis of Cys232 to Ser232 in *E. coli* SdiA
*sdiA*C232S-R	GCGGCCGCGTAAGAGGCAACCTGGG	
*ftsQ*P2-F	TCCAGTGTGGGAATGTCAAAAGTAGTAGCAGAAAATGCTCTACAAGATGCATTA	synthesis of 54 bp in length of the *E. coli ftsQ*P2 promoter region
*ftsQ*P2-R	AATGCATCTTGTAGAGCATTTTCTGCTACTACTTTTGACATTCCCACACTGGAA	
uvrY-pro-F	GACCAATAAATATTTTTATCATGAATG	amplification of 339 bp in length of the *E. coli uvrY* promoter region
uvrY-pro-R	CAACAAGTAGAACGTTGATCAAAG	