Table S1. Selected MAD Statistics for $P3_2$ BldD CTD-c-di-GMP Data, Related to Figures 4 and 5

Wavelength (Å)	0.979 (Peak)	0.979 (Inflection)	0.923 (Remote)
Space group	$P3_2$	$P3_2$	$P3_2$
a, b, c (Å)	a=b=86.5, c=151.5	a=b=86.5, c=151.5	a=b=86.5, c=151.5
α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120
Resolution (Å)	75.4-2.28	75.4-2.28	75.4-2.28
R_{sym} (%)[a]	5.7 (18.9)[b]	4.6 (44.3)	5.0 (30.9)
I/σ(I)	15.5 (3.0)	14.2 (2.3)	13.8 (2.5)
Total reflections (#)	317916	118771	72566
Unique reflections (#)	59112	36721	9226

[a]$R_{sym} = \Sigma\Sigma|I_{hkl}-I_{hkl(j)}|/ \Sigma I_{hkl}$, where I_{hkl} is observed intensity and $I_{hkl(j)}|$ is the final average value of intensity.

[b]Values in parentheses are for the highest resolution shell.

Table S2. Selected Crystallographic Refinement Statistics for BldD CTD-c-di-GMP Structures, Related to Figures 4, 5, and 7

Complex	*S. venezuelae* BldD C-domain-(c-di-GMP) (form 1)	*S. venezuelae* BldD C-domain-(c-di-GMP) (form 2)	*S. venezuelae* BldD C-domain-(c-di-GMP) (form 3)	*S. coelicolor* BldD C-domain-(c-di-GMP)
Data Collection				
Space group	$P3_2$	$P2_12_12$	$C222_1$	$C222_1$
a, b, c (Å)	a=b=86.5, c=151.5	a=136.5, b=98.8, c=68.8	a=36.2, b=95.5, c=99.5	a=36.9, b=95.5, c=100.1
α, β, γ (°)	90, 90, 120	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	75.8-1.95	98.8-2.33	99.6-1.75	34.6-2.25
R_{sym} (%)[a]	5.7 (57.9)[b]	4.6 (44.3)	4.1 (38.5)	5.0 (30.9)
I/σI	11.4 (1.9)	14.2 (2.3)	14.6 (2.6)	13.8 (2.5)
Total reflections (#)	322678	118771	57815	72566
Unique reflections (#)	90983	36721	17271	9226
Refinement				
R_{work}/R_{free} (%)[c]	19.8/24.4	22.6/26.9	19.8/21.9	22.6/25.6
Ramachandran analysis				
Most Favored (%/#)	91.8/752	84.3/452	92.1/117	94.4/117
Add. Favored (%/#)	8.7/67	14.7/79	7.910	5.6/7
Allowed (%/#)	0.0/0	0.9/5	0.0/0	0.0/0
Outlier (%/#)	0.0/0	0.0/0	0.0/0	0.0/0
Rmsd				
Bond lengths (Å)	0.020	0.017	0.008	0.017
Bond angles (Å)	1.99	2.04	1.32	2.03

[a]$R_{sym} = \Sigma\Sigma|I_{hkl}-I_{hkl}(j)|/ \Sigma I_{hkl}$, where I_{hkl} is observed intensity and $I_{hkl}(j)|$ is the final average value of intensity.

[b]Values in parentheses are for the highest resolution shell.

[c]$R_{work} = \Sigma||F_{obs}| - |F_{calc}||/\Sigma|F_{obs}|$ and $R_{free} = \Sigma||F_{obs}| - |F_{calc}||/\Sigma|F_{obs}|$; where all reflections belong to a test set of 5% randomly selected data.

Table S3. Strains and Plasmids Used in this Study, Related to Experimental Procedures

	Relevant genotype/comments	Source or reference
Strains		
S. venezuelae		
ATCC 10712	Wild type	(Bibb et al., 2012)
SV70	*attB*$_{\Phi BT1}$::pIJ10350 (*ermEp*- cdgB)*; Hyg^R	This study
SV71	*attB*$_{\Phi BT1}$::pIJ10659 (*ermEp* - yhjH*); Hyg^R	This study
SV77	*ΔbldD::apr*; Apr^R (Redirect)	This study
SV74	*ΔbldD::apr*; Apr^R (SV1-transduction)	This study
SVNT6	*attB*$_{\Phi BT1}$::pSVNT5 (*ermEp*- cdgB*-G472A*)*; Hyg^R	This study
SVNT8	*attB*$_{\Phi BT1}$::pSVNT4 (*ermEp*- yhjH*-E48A*)*; Hyg^R	This study
SVNT9	*ΔbldD::apr*; Apr^R *attB*$_{\Phi BT1}$::pIJ10350 (*ermEp*- cdgB)*; Hyg^R	This study
SVNT12	*ΔbldD::apr*; Apr^R *attB*$_{\Phi BT1}$::pSVNT7 (pMS82-*bldD* R114D, D116R, R125D, D128R); Hyg^R	This study
E. coli		
ET12567/pUZ8002	*dam*, *dcm*, *hsd*, Kan^R, Cm^R	(Paget et al., 1999)
BW25113/pIJ790	(Δ(*araD-araB*)*567*, Δ*lacZ4787*(::*rrnB-4*), *lacIp-4000*(lacI^Q), λ-, *rpoS369*(Am), *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR514*; Cm^R	(Datsenko and Wanner, 2000)
BL21(DE3)/pLysS	F– *ompT hsdS*(r$_{B-}$ m$_{B-}$) *gal dcm* λ(DE3), Cm^R	Promega
DH5α	F$^-$ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17(r$_K^-$ m$_K^+$), λ–	(Hanahan, 1985)
Plasmids		
pIJ773	Plasmid template for amplification of the *apr-oriT* cassette for 'Redirect' PCR-targeting	(Gust et al., 2003)
pIJ790	Modified λ RED recombination plasmid [*oriR101*] [*repA101*(ts)] *araBp-gam-be-exo*, Cm^R	(Gust et al., 2003)
pUZ8002	RP4 derivative with defective oriT, Kan^R	(Paget et al., 1999)
pET15b	T7 expression vector, Amp^R	Novagen
pIJ10257	Plasmid integrating at the ϕBT1 *attB* attachment site containing the constitutive *ermEp** promoter, Hyg^R	(Hong et al., 2005)
pIJ10350	pIJ10257 carrying *cdgB* from *S.coelicolor*	(Tran et al., 2011)
pIJ10659	pIJ10257 carrying N-terminally codon optimised *yhjH* from *E. coli*	This study
pIJ10663	pET15b-*bldD*-Full	This study
pIJ10661	pET15b-*bldD*-NTD	This study
pIJ10660	pET15b-*bldD*-CTD	This study
pIJ10674	pET15b-*bldD* R114D, D116R, R125D, D128R	This study
pIJ10676	pET15b-*bldD* R125D, D128R	This study
pIJ10677	pET15b-*bldD* R114D, D116R	This study

pIJ10668	pET15b-*bldD*-CTD L92M	This study
pIJ10678	pET15b-*bldD*-CTD L92M, I135M	This study
pSVNT4	pIJ10257 carrying N-terminally codon optimised *yhjH*-E48A from *E. coli*	This study
pSVNT5	pIJ10257 carrying *cdgB*-G472A from *S.coelicolor*	This study
pSVNT7	pMS82-*bldD* R114D, D116R, R125D, D128R	This study

Table S4. Oligonucleotides Used in this Study, Related to Experimental Procedures

Nucleotides in italics indicate restriction sites and nucleotides in bold represent mutations introduced

Oligonucleotide	Sequence
Oligonucleotides used for cloning of *bldD*-full, *bldD*-NTD and *bldD*-CTD into pET15b	
bldDSVfull-NdeI-fw	GGTGGT*CATATG*TCCAGCGAATACGCAAAGCAGC
bldDSVfull-BamHI-rev	CTCCTC*GGATCC*TCAGTTCTCCTCGTGGGCGACG
bldDSVntd-BamHI-rev	CTCCTC*GGATCC*TCAGGCGGCCCCGCCCGGAGTCG
bldDSVctd-NdeI-fw	GGTGGT*CATATG*GAGCCGCCGCCGAAGCTCGTCC
Oligonucleotides used for mutagenesis of *bldD*	
bldD-L92M-fw	CTGGAGCGC**ATG**GCGCACGTC
bldD-L92M-rev	GACGTGCGC**CAT**GCGCTCCAG
bldD-I135M-fw	CTGGCCGTG**ATG**TACGACCAGTC
bldD-I135M-rev	GACTGGTCGTA**CAT**CACGGCCAG
bldD R114D, D116R fw	ATCCAGTCGCAG**GAC**GGC**CGC**TACAACGGCAAG
bldD R114D, D116R rev	CTTGCCGTTGTA**GCG**GCC**GTC**CTGCGACTGGAT
bldD R125D, D128R fw	GTGCTGTCGATC**GAC**CAGGAC**CGT**CTGCGCACCCTG
bldD R125D, D128R rev	CAGGGTGCGCAG**ACG**GTCCTG**GTC**GATCGACAGCAC
Oligonucleotides used for cloning of *yhjH* and *cdgB* into pIJ10257	
yhjH-NdeI-fw	GGTGGT*CATATG*ATCCGCCAGGTCATCCAGCGCATCTCCAACCCTGAAG CAAGCATCGAGAGC
yhjH-HindIII-rev	CGT*AAGCTT*TCATAGCGCCAGAACCGCCGTA
cdgBSCO-NdeI-fw	CGTGGT*CATATG*GAGACCGACTCGGAGCC
cdgBSCO-HindIII-rev	CGT*AAGCTT*TCATCCGGCGCGGCGGTGCTG
Oligonucleotides used for generating DNA fragments for gel retardation assays	
pbldMSV-pcr-fw	CTAGCCACAGACACCGCG
pbldMSV-pcr-rev	CGACGGTCACTCGAAAGAG
pwhiGSV-pcr-fw	GTTCGAAGATGTGGCCGAC
pwhiGSV-pcr-rev	GCGTTGCCTTGAGCCGTTC
Oligonucleotides used for amplification of the *oriT-apr* cassette with *bldD*-specific extensions	
bldD sense	GCGCAGCCGCATGTCGTCACAGCGTCCGGGAGCGTTATGATTCCGGGG ATCCGTCGACC
bldD disruption R2	TCTGCTGGGTCCCCGTAAGGGGTTCAGTTCTCCTCGTGGTGTAGGCTGG AGCTGCTTC

Oligonucleotides used for verification of the *bldD* mutant and for cloning into pMS82

bldDSV-HindIII-pMS82-fw	GGTGGT*AAGCTT*GAAGAAACGGACCTCCTTCTCC
bldDSV-KpnI-pMS82-rev	AGTAGT*GGTACC*GTCCGTAGACGTCACCGGCAGTCG

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