

Supplemental Data for "Natural diversity provides a broad spectrum of cyanobacteriochrome-based diguanylyl cyclases"

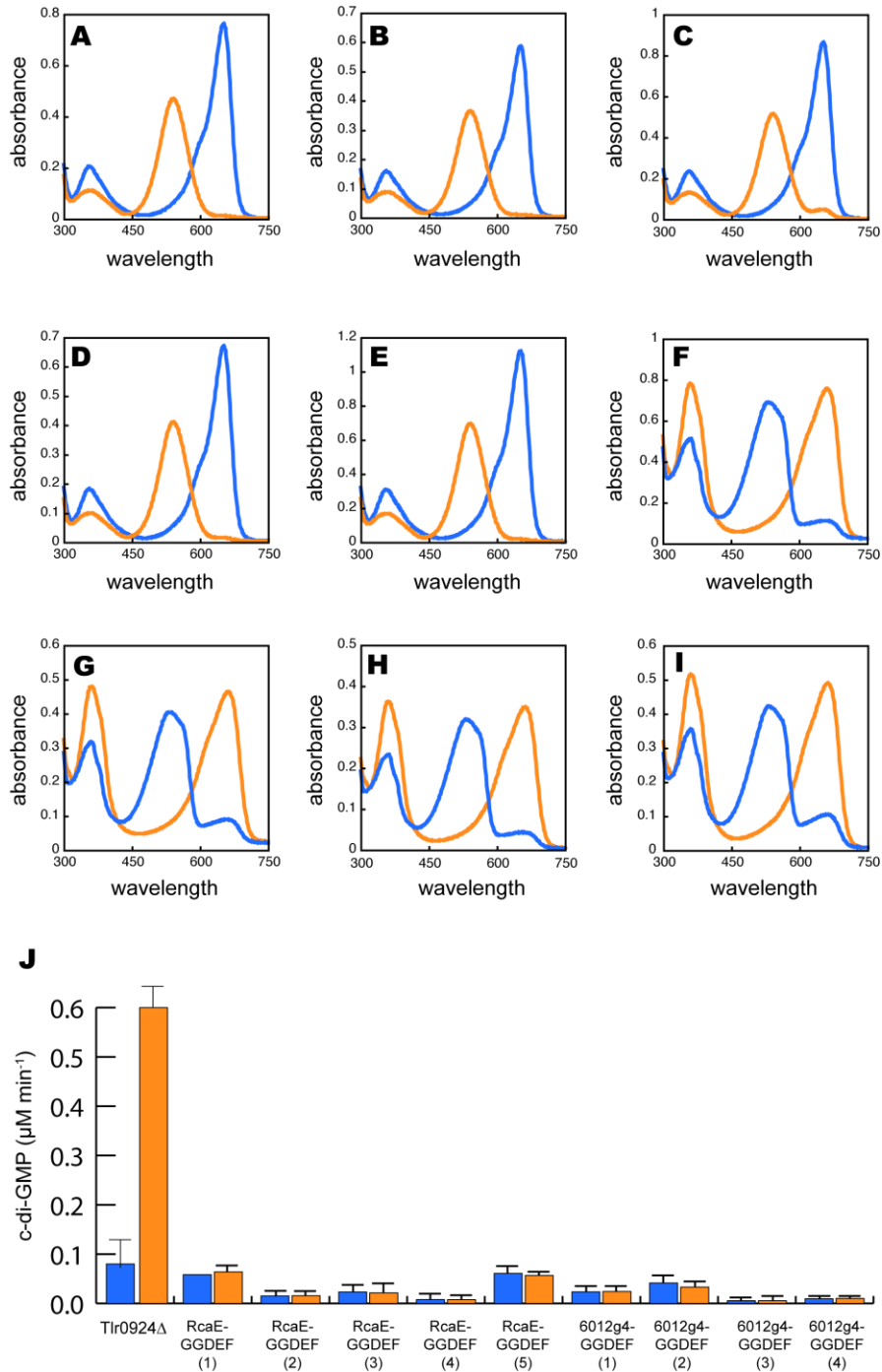
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Five figures, one table and one citation.

Supplemental Figure S1. Spectral analysis of selected fusion CBCR-DGC proteins. Absorbance spectra of: (A) NpR6012g4-GGDEF(1), (B) NpR6012g4-GGDEF(2), (C) NpR6012g4-GGDEF(3), (D) NpR6012g4-GGDEF(4), (E) NpR6012g4-GGDEF(5), (F) RcaE-GGDEF(1), (G) RcaE-GGDEF(2), (H) RcaE-GGDEF(3), (I) RcaE-GGDEF(4) (J) DGC enzyme assay data; values (\pm SD, $n = 3$) are shown. For all panels, *15Z* = blue trace/bar, *15E* = orange trace/bar).



Supplemental Figure S2. CBCR GAF domain alignment. Amino acid sequence alignment of CBCR (GAF) domain of CBCR-DGC proteins. Bold lettering indicates proteins examined in this work. Blue highlighting denotes canonical '1st cysteine' residue. Yellow highlighting denotes DXCF motif. Red highlighting denotes 2nd cysteine residue of Lyn8106_0097Δ and Lyn.aest_0230Δ.

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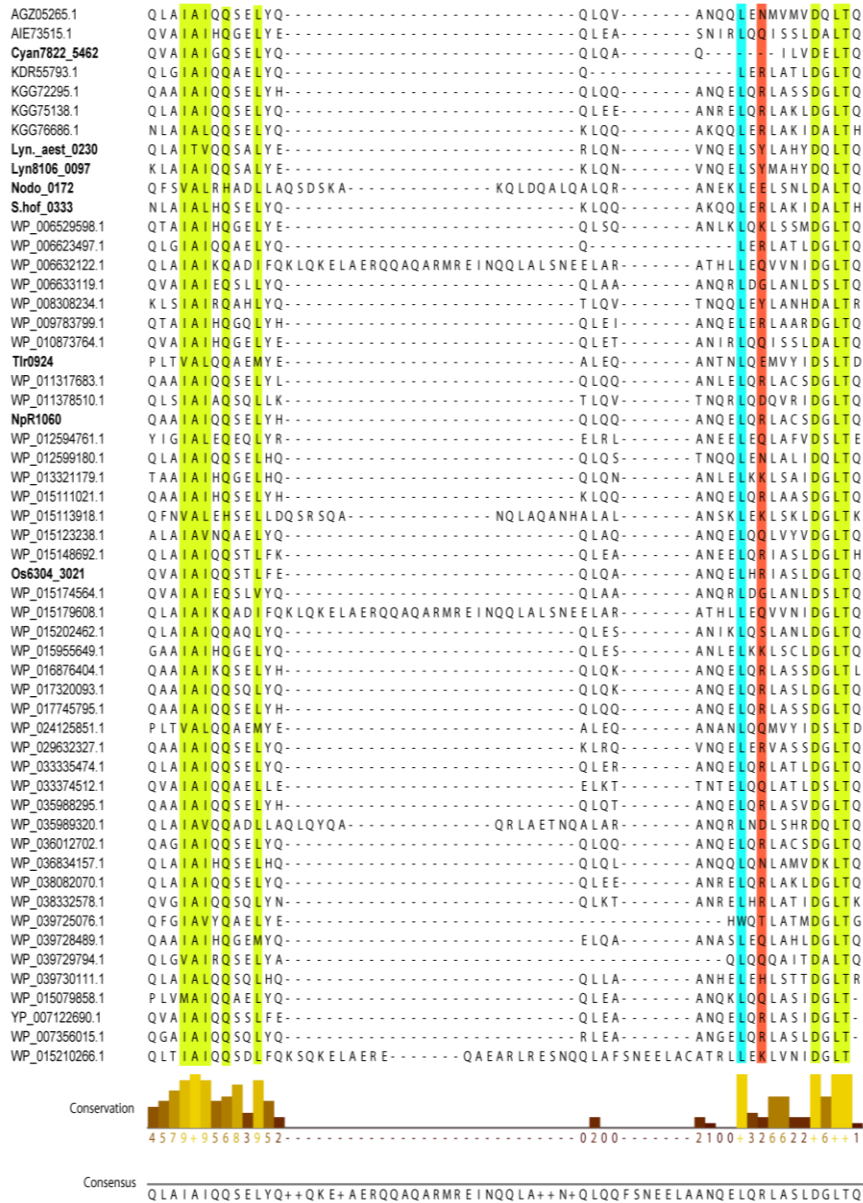
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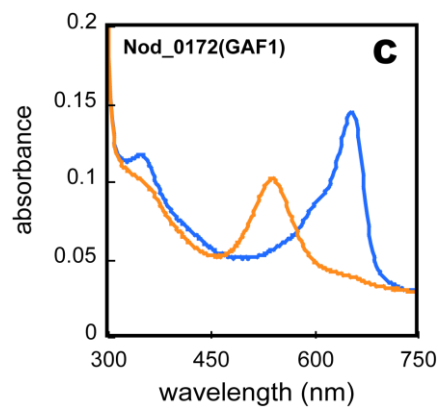
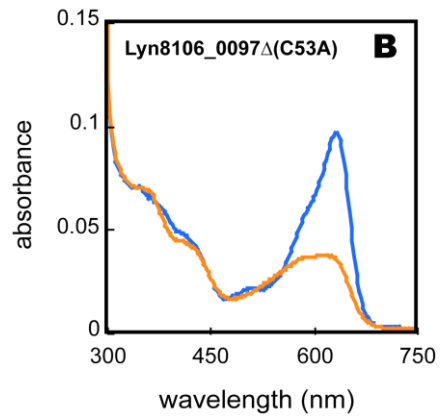
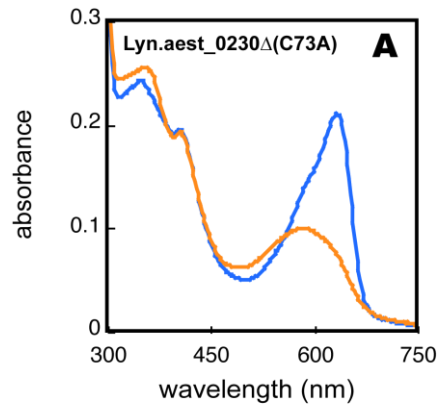
Supplemental Figure S3. DGC GGDEF domain alignment. Amino acid sequence alignment of GGDEF domain of CBCR-DGC proteins. Bold lettering indicates proteins examined in this work. Blue highlighting denotes RxxD inhibition site motif. Yellow highlighting denotes 'GGDEF' DGC active site motif.

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WP_039729794 DGLTQIANRRCFDHLERHWQNLARQKRLPSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
WP_039725076 DGLTQIANRRCFDHLERHWQNLARQKRLPSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
WP_011378610 DGLTQIANRRCFDHLERHWQNLARQKRLPSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
WP_017745795 DGLTQIANRRCFDHLERHWQNLARQKRLPSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
WP_029632327 DGLTQIANRRCFDHLERHWQNLARQKRLPSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
NgR1060 DGLTQIANRRCFDHLERHWQNLARQKRLPSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
WP_011317883 DGLTQIANRRCFDHLERHWQNLARQKRLPSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
WP_036012702 DGLTQIANRRCFDHLERHWQNLARQKRLPSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
WP_035988295 DGLTQIANRRCFDHLERHWQNLARQKRLPSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
DALTHVANRRRFDHYLQEQWRRLTRERKPLSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
S.hof_0333 DALTHVANRRRFDHYLQEQWRRLTRERKPLSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
KGG76686 DALTHVANRRRFDHYLQEQWRRLTRERKPLSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
WP_007356015 DALTHVANRRRFDHYLQEQWRRLTRERKPLSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
WP_015174564 DALTHVANRRRFDHYLQEQWRRLTRERKPLSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
WP_006633119 DALTHVANRRRFDHYLQEQWRRLTRERKPLSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS
O6s304_3201 DGLTQIANRRCFDHLERHWQNLARQKRLPSL LDCIDYFKYNDYVYHGGDCLTVQAALKASASD LVARYGGEEFVVL PNTDNFGAIVAQAQVIRKLELRPHRASVYVTVS

Supplemental Figure S4. CBCR-DGC linker alignment. Amino acid sequence alignment of linker region between CBCR and GGDEF domains. Bold lettering indicates proteins further discussed in this work. Blue highlighting denotes conserved leucine residue discussed in previous work (Blain-Hartung et al., 2017). Red highlighting denotes conserved polar, hydrophilic residue discussed in text. Yellow highlighting denotes residues that are conserved in $\geq 90\%$ of observed proteins.



Supplemental Figure S5. Spectroscopic characterization of 2nd cysteine mutant constructs of novel R/B CBCR proteins and the GAF1-only construct, Nod_0172(GAF1). (A) Lyn.aest_0230 Δ (C73A), (B) Lyn8106_0097 Δ (C53A), (C) Nod_0172(GAF1). 15Z, blue traces; 15E, orange traces.



Supplemental Table S1: Accession information for proteins in this study¹

Protein	Genbank accession	DOE-IMG locus tag	Organism
Os6304_3021	AFY82617	Oscil6304_3021	<i>Oscillatoria acuminata</i> PCC 6304
S.hof_0333	KYC42878	WA1DRAFT_10042	<i>Scytonema hofmannii</i> PCC 7110
NpR1060	ACC79786	Npun_R1060	<i>Nostoc punctiforme</i> PCC 73102
Lyn8106_0097	EAW38947	L8106_01492	<i>Lyngbya</i> sp. PCC 8106
Lyn.aest_0230	ERT07933	M595_2050	<i>Lyngbya aestuarii</i> BL J
Cyan7822_5462	ADN17337	Cyan7822_5462	<i>Gloeotheca verrucosa</i> PCC 7822 ²
Nod_0172	WP_155523487	Lepto7104DRAFT_1266	<i>Nodosilinea nodulosa</i> PCC 7104

1. Current Genbank accessions, DOE-IMG locus tags, and current organism/strain designations are provided for each newly characterized protein in this study (Fig. 3).

2. *Gloeotheca verrucosa* PCC 7822 is also known as *Cyanothece* sp. PCC 7822.

Supplemental Citation

Blain-Hartung MD, Rockwell NC, Lagarias JC (2017) Light-regulated synthesis of cyclic-di-GMP by a bidomain construct of the cyanobacteriochrome Tlr0924 (SesA) without stable dimerization. *Biochemistry* 56: 6145-6154.