Supporting Information

A Flavin Cofactor-Binding PAS Domain Regulates C-di-GMP Synthesis in *Ax*DGC2 from *Acetobacter xylinum*

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Table S1. Absorption maxima for the wild type and mutant *Ax*DGC2.

Protein	Cofactor	Wavelength (nm)
*Ax*DGC2	FAD	375, 451, 480
H62A	FAD	341, 427
N66A	FAD	375, 455, 480
N94A	-	-
R125A	FAD	375, 449, 476
D217A	FAD	375, 451, 480

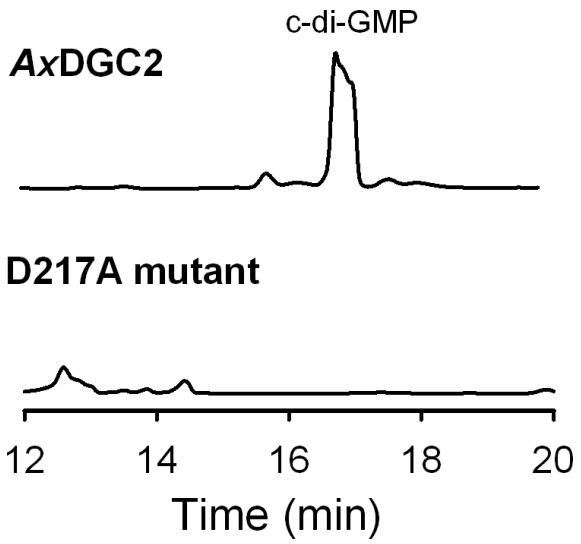

Figure S1. HPLC analysis of the binding of c-di-GMP by the GGDEF domain at the I-site shows that the D217A mutation abolishes c-di-GMP binding.

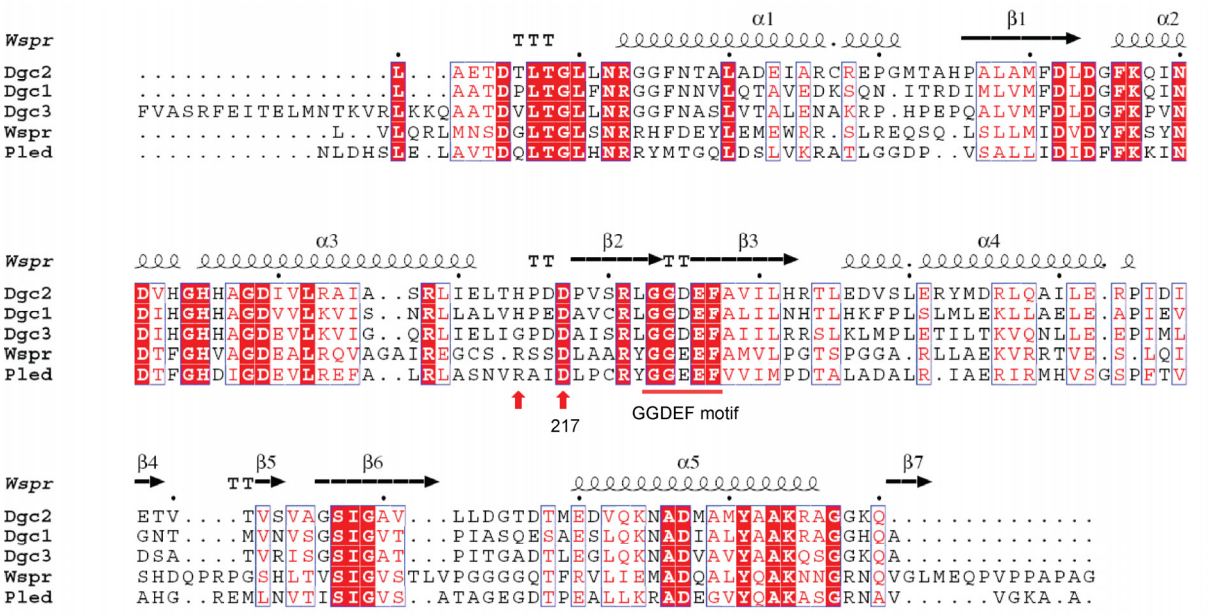

Figure S2. Sequence alignment of the GGDEF domains of *Ax*DGC1, 2, 3 and two orthodox GGDEF domains of WspR and PleD. The conserved GGDEF motif and the residues (including Asp^{217}) in the I-site are highlighted by the underlining bar and arrows.

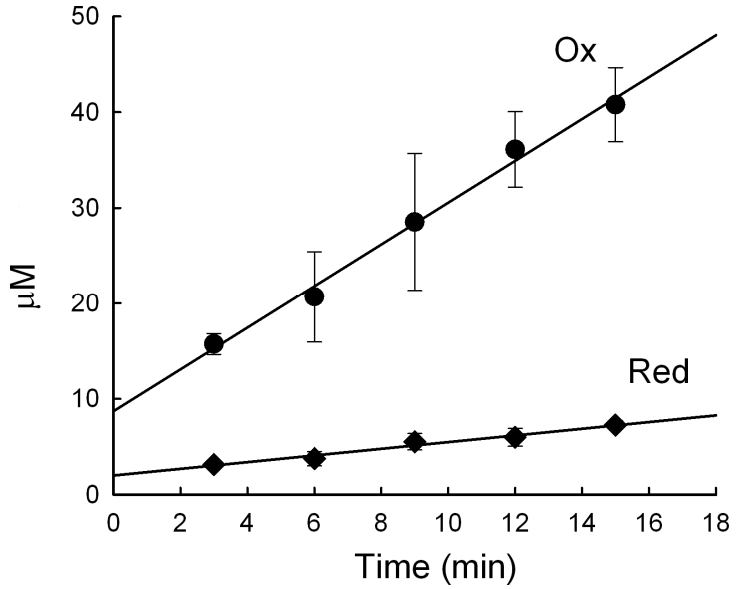

Figure S3. Comparison of the diguanylate cyclase activity of the oxidized and reduced forms of *Ax*DGC2 with the use of the xanthine oxidase system (V_0(Ox) = 0.38 μM/min; V_0(Red) = 0.07 μM/min). For the reduced form, 15 μM *Ax*DGC2, 400 μM xanthine and 1.5 μM Benzyl viologen were incubated in 100 mM Tris-Cl (pH8.0), 50 mM KCl buffer. The anaerobiosis was established by flushing the anaerobic chamber with N_2. Catalytic amount of xanthine oxidase was added in to initiate the reduction, which was monitored by UV spectrometry. For the oxidized form, the electron transfer mediator benzyl viologen was left out from the reaction mixture and the presence of oxidized FAD was confirmed by UV-Vis spectroscopy. Reaction buffer of DGC activity assay was 100 mM Tris-Cl (pH8.0), 50 mM KCl, 10 mM $MgCl_2$. Final substrate concentration was 100 μM and the enzyme concentration was 2 μM.

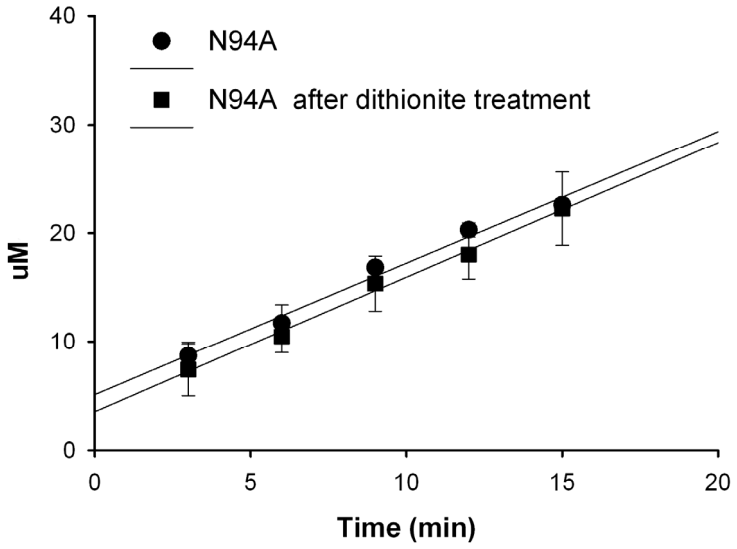

Figure S4. Comparison of the catalytic efficiency of the FAD-free N94A mutant in the presence or absence of sodium dithionite under anaerobic conditions. The similarity between the two forms indicated that the dithionite does not have a noticeable effect on the diguanylate cyclase activity when there is no FAD in the PAS domain. The reaction conditions are similar to the ones described in Material and Methods.

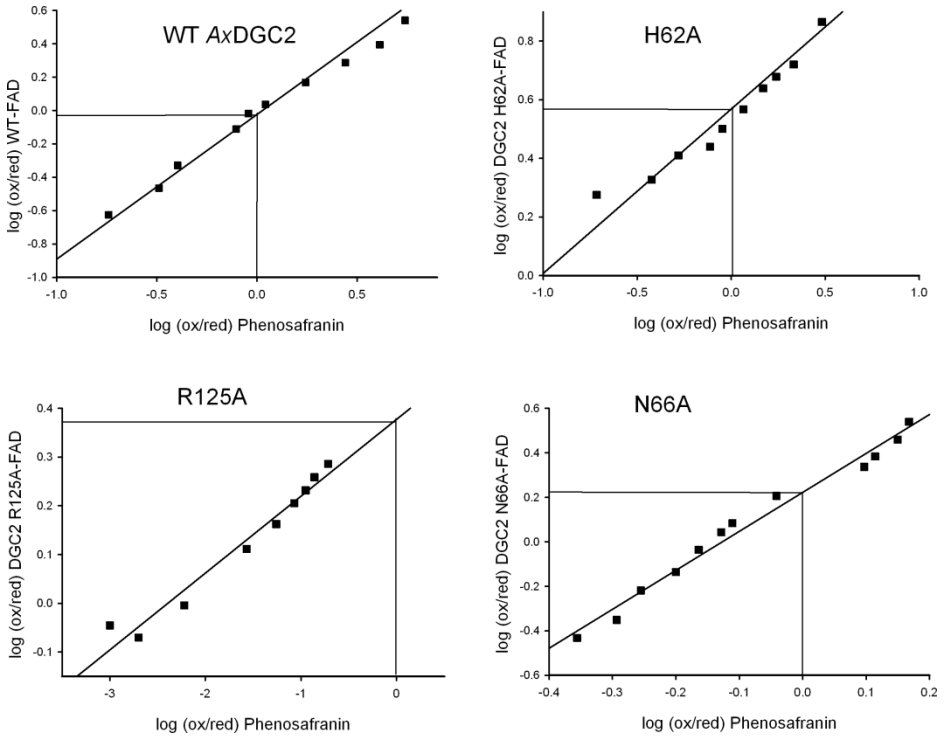

Figure S5. Nernst plots of log(ox/red) phenosafranin as a function of log (ox/red) *Ax*DGC2-FAD. The redox potentials of the FAD in *Ax*DGC2 and the mutants were obtained from the y intercept, where log (ox/red) phenosafranin equals zero.

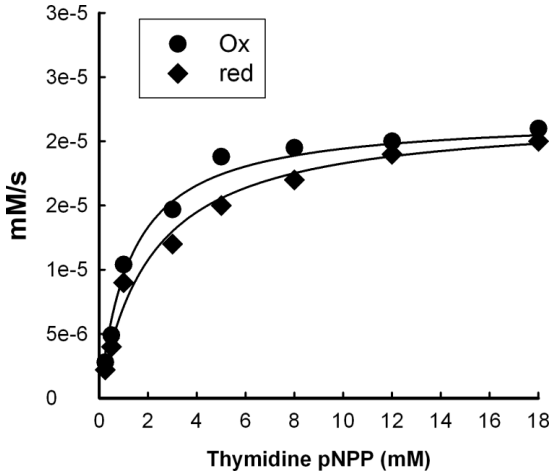

Figure S6. Comparison of the phosphodiesterase activity of the oxidized and reduced forms of *Ax*DGC2 with the use of 1 mM sodium dithionite. Reaction buffer was 50 mM Tris-Cl (pH8.0), 25 mM $MgCl_2$.

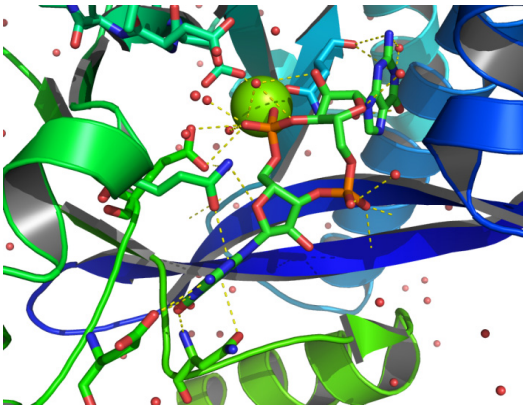

Figure S7. Binding of the cyclic-di-GMP by the EAL domain of TdEAL (PDE entry: 3II8). Cyclic-di-GMP and the residues involved in substrate binding are represented by the sticks. The substrate-binding residues are conserved in the EAL domain of *Ax*DGC2.

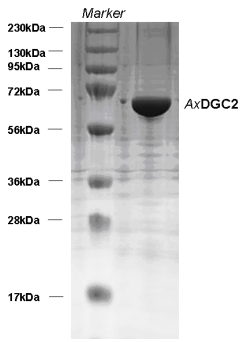

Figure S8. SDS-PAGE gel of purified *Ax*DGC2.