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Supplemental Information for

The *D*-ring, not the *A*-ring, rotates in *Synechococcus* OS-B' phytochrome

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This PDF file includes:

Supplemental Figures S1-S3

Supplemental Figure S1 Sequence of SyB.Cph2(GAF):H₆ with optimized codon usage for expression in E. coli as used in this work.



Supplemental Figure S2 Enlarged contour plots of the DARR spectra of u-[¹³C,¹⁵N]-PCB-SyB.Cph2(GAF) as P630 with mixing times of 5 (red) and 50 (purple) ms. Two 1D traces of the 2D spectra along ω_1^- (right) and ω_2^- dimension (top) are shown with the assignments of the ¹³C peaks. The indirectly bonded ¹³C–¹³C correlations are indicated by arrows and labeled in green. Resonances of the natural abundance glycerol carbons are indicated by asterisks.



Supplemental Figure S3 *Enlarged contour plots of the DARR spectra of u-[¹³C,¹⁵N]-PCB-SyB.Cph2(GAF) as P690 with mixing times of 5 (orange)* and 50 (cyan) ms. Two 1D traces of the 2D spectra along ω_1 - (right) and ω_2 -dimension (top) are shown with the assignments of the ¹³C peaks. The indirectly bonded ¹³C–¹³C correlations are indicated by arrows and labeled in green. Resonances of the natural abundance glycerol carbons are indicated by asterisks.