Supporting Information for

Ligation of D1-His332 and D1-Asp170 to the Manganese Cluster of Photosystem II from *Synechocystis* Assessed by Multifrequency Pulse EPR Spectroscopy

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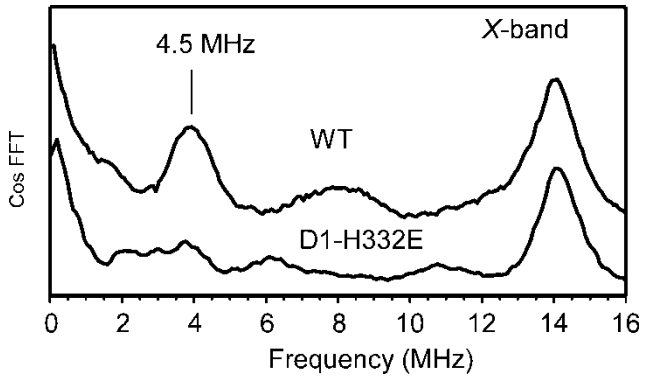

Figure S1. Comparison of cosine-backfilled Fourier transformed two-pulse ESEEM spectra for WT* and D1-H332E mutant of PSII from *Synechocystis* sp. PCC 6803 obtained at 0.3420 mT with microwave frequency 9.235 GHz. Adapted from Figure 5 in Debus *et al*. 2001 *Biochemistry.*

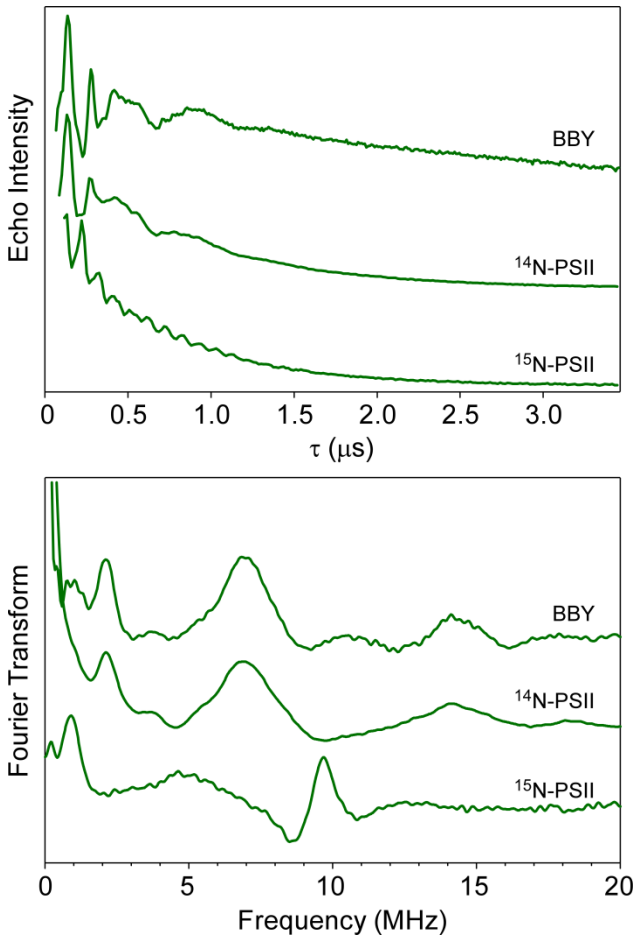

Figure S2. Time-domain (top) and corresponding cosine-backfilled Fourier transformed (bottom) K_a-band two-pulse ESEEM spectra of BBY, and ^{14}N-PSII and ^{15}N-PSII from *Synechocystis* obtained at g = 1.98. Instrument settings: ν_{MW} = 30.757 GHz, B_0 = 1.1079 T, $\pi/2$ = 10 ns, $\Delta\tau$ = 15 ns, repetition time = 5 ms, T = 4.5 K.

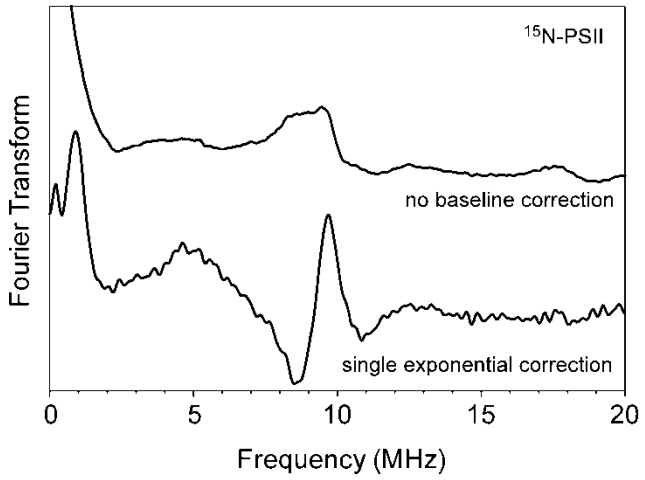

Figure S3. Effect of exponential baseline subtraction on Fourier transformed K_a-band two-pulse ESEEM of ^{15}N-PSII. Spectrometer settings same as those listed in caption of Figure S2.

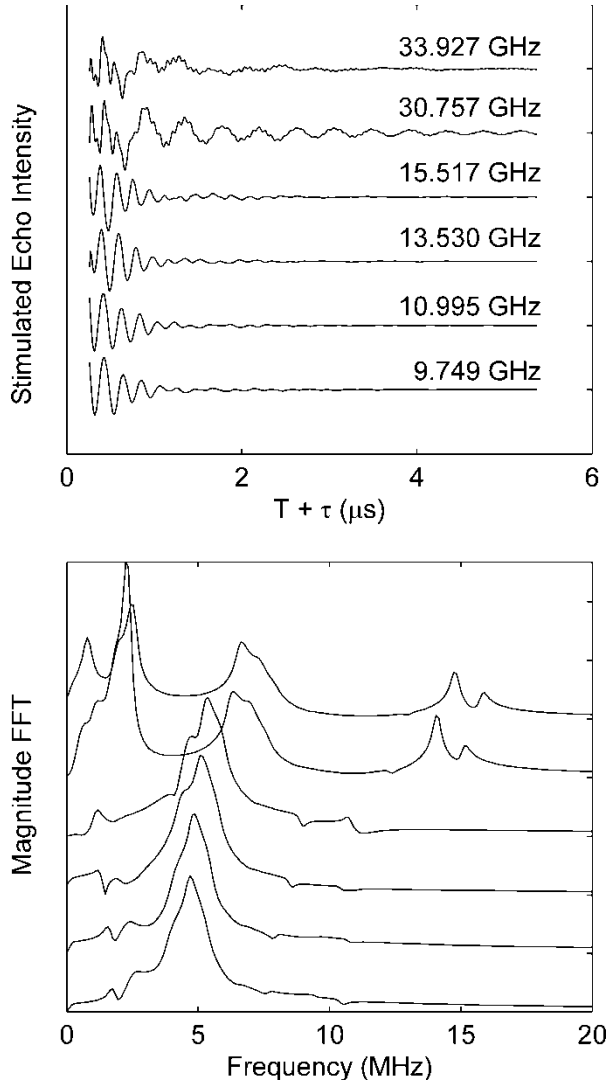

Figure S4. Multifrequency (B_0 corresponds to g = 1.98) three-pulse ESEEM spectra simulated using ^{14}N magnetic parameters given in Table 1.

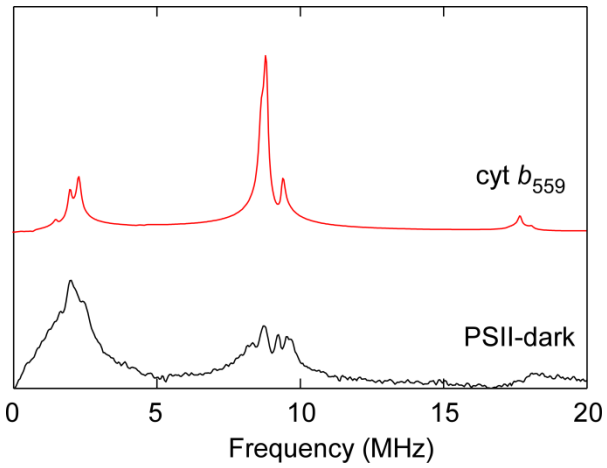

Figure S5. Comparison of *Q*-band three-pulse ESEEM spectrum of dark-adapted ^{15}N-PSII and a simulation of the ^{15}N-labeled cyt b_{559}. Spectrometer settings are same as those given in the caption of Figure 3.

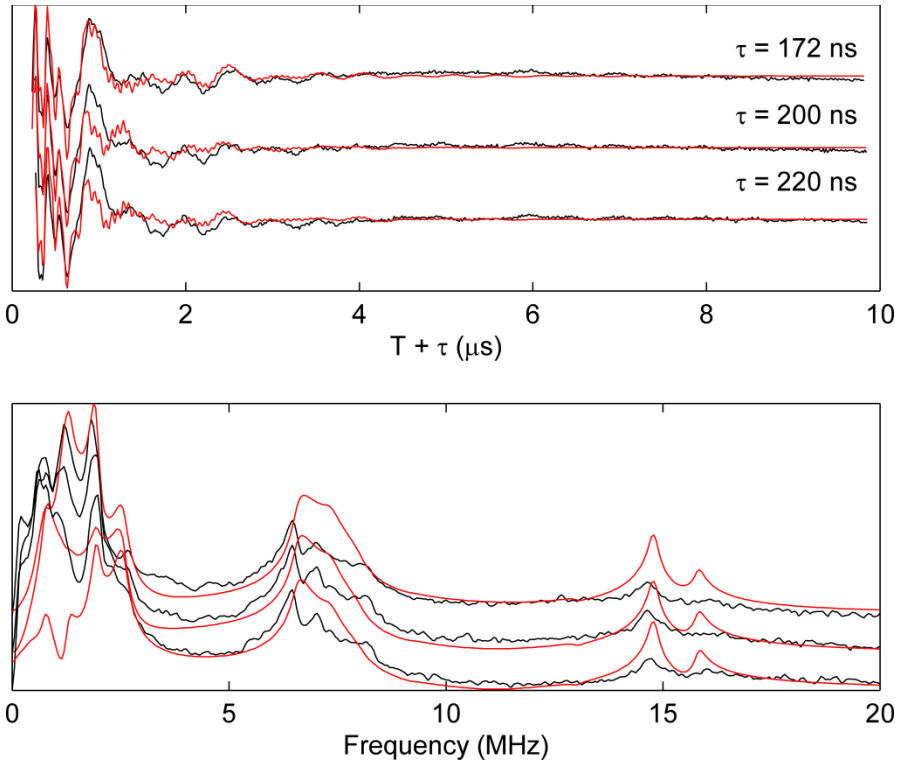

Figure S6. *τ*-dependence of *Q*-band three-pulse ESEEM spectra of light-*minus*-dark ^{14}N-PSII with corresponding simulations obtained using parameters given in Table 1. Spectrometer settings are same as those given in caption of Figure 3.

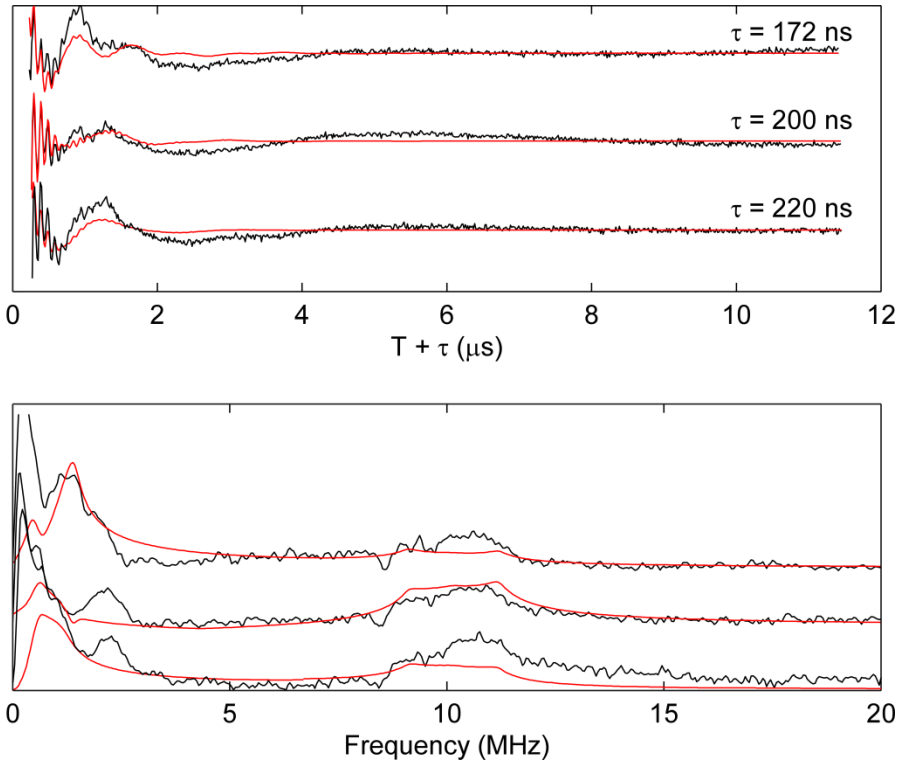

Figure S7. *τ*-dependence of *Q*-band three-pulse ESEEM spectra of light-*minus*-dark ^{15}N-PSII with corresponding simulations obtained using parameters given in Table 1. Spectrometer settings are same as those given in caption of Figure 3.

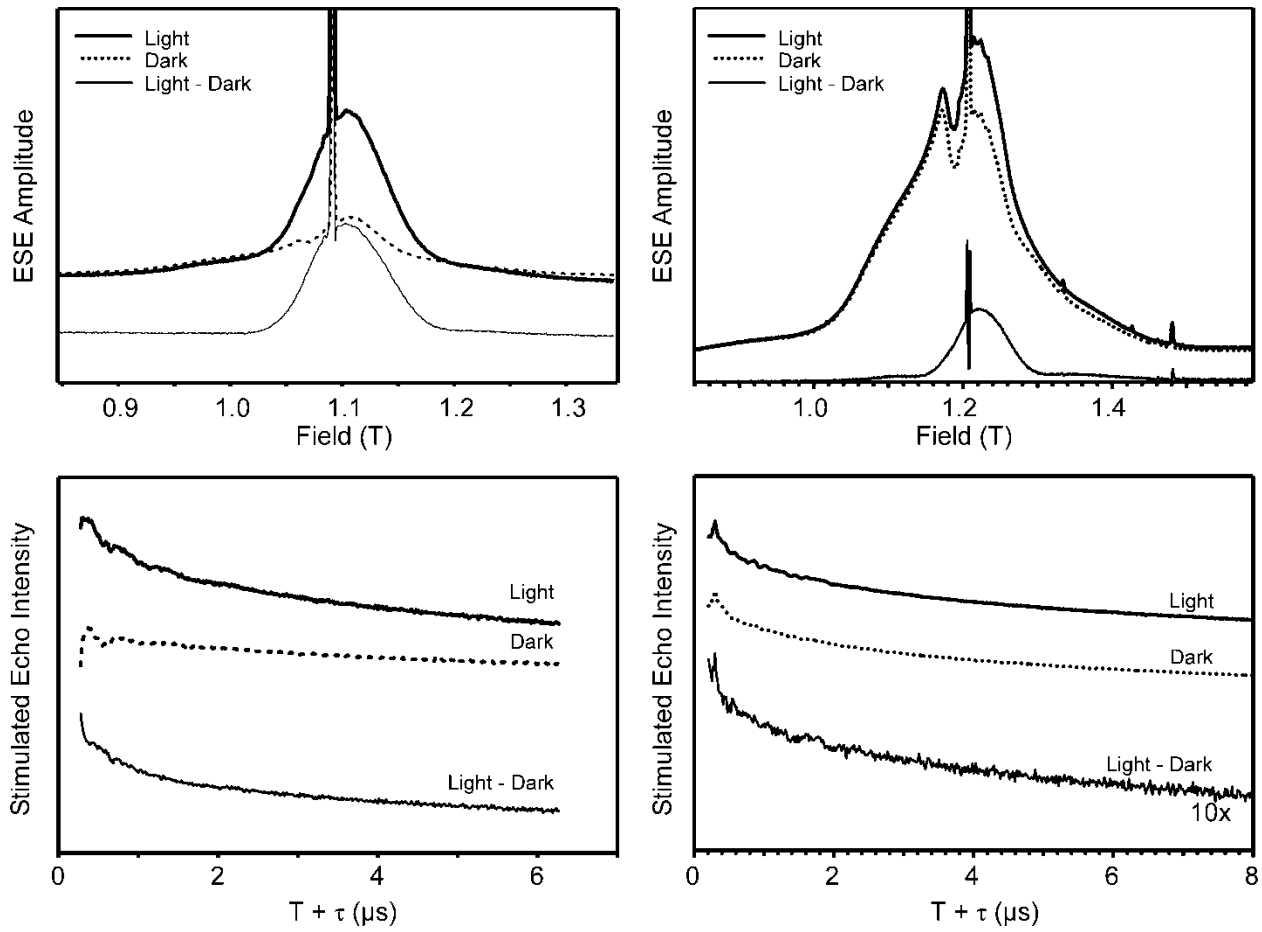

Figure S8. Light, dark, and difference ESE-EPR spectra (top) for D1-H332E acquired at K_a-(ν_{MW} = 30.562 GHz, left) and Q-band (ν_{MW} = 33.859 GHz, right) and corresponding three-pulse ESEEM spectra (bottom). Additional instrument settings for K_a-band ESE-EPR: $\pi/2$ = 30 ns, τ = 220 ns, ΔB_0 = 1 mT, repetition time = 5 ms, T = 5.0 K; K_a-band three-pulse ESEEM:B_0 = 1.1063 T, $\pi/2$ = 10 ns, τ = 210 ns, ΔT = 15 ns, repetition time = 5 ms; Q-band ESE-EPR: $\pi/2$ = 32 ns, τ = 200 ns, ΔB_0 = 1 mT, repetition time = 5 ms, T = 4.5 K; Q-band three-pulse ESEEM: B_0 = 1.2190 T, $\pi/2$ = 16 ns, τ = 128 ns, ΔT = 16 ns.