Optimization of FeMoco Maturation on NifEN

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Supporting Information

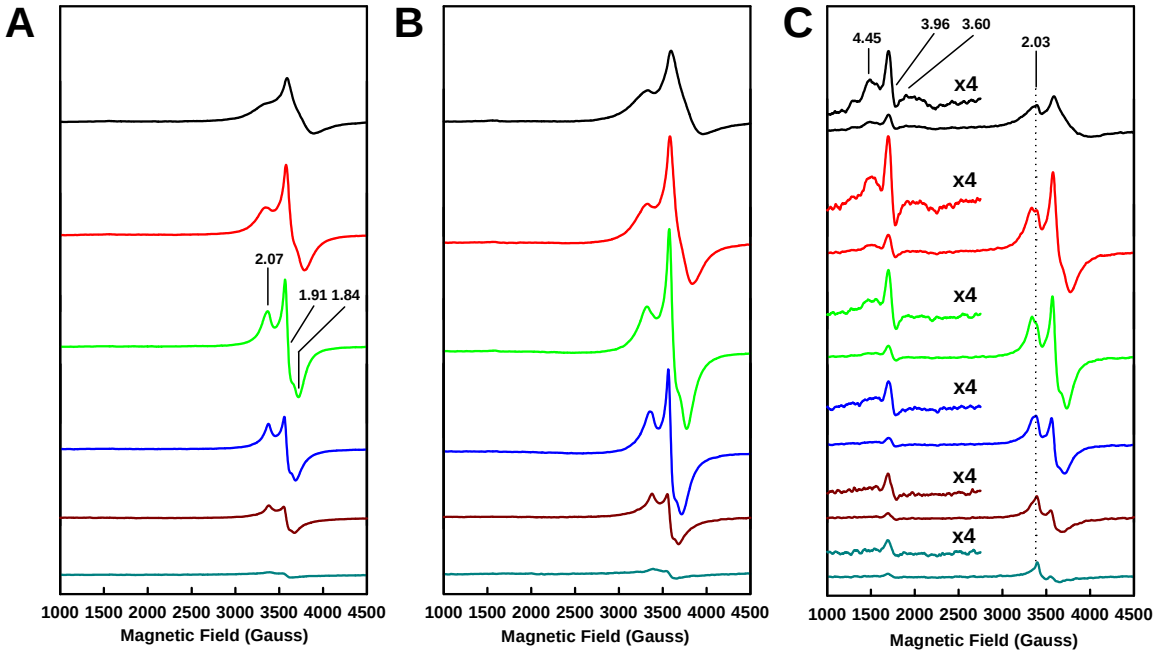

Figure S1. Temperature-dependency of EPR spectra of Δ*nifB* NifEN (A), NifEN^{Precursor} (B) and NifEN$^{\text{“FeMoco”(20 mM)}}$ (C) in dithionite-reduced states. The precursor-free Δ*nifB* NifEN (A) contains two permanent [Fe_4S_4] clusters at the α/β subunit interface, which gives rise to an S = 1/2 signal; whereas NifEN^{Precursor} (B) contains, in addition to the permanent [Fe_4S_4] clusters, a FeMoco precursor that gives rise to a signal that overlaps with the S = 1/2 signal of the [Fe_4S_4] clusters, which accounts for the differences in signal intensity and temperature- and power-dependency between the EPR spectra of NifEN^{Precursor} and Δ*nifB* NifEN.[1] In contrast to both Δ*nifB* NifEN and NifEN^{Precursor}, NifEN$^{\text{“FeMoco”(20 mM)}}$ (C) contains a “FeMoco”, which gives rise to additional features in both S = 3/2 and S = 1/2 regions. The S = 3/2 signals of NifEN$^{\text{“FeMoco”(20 mM)}}$ are enlarged, and the g values are given.

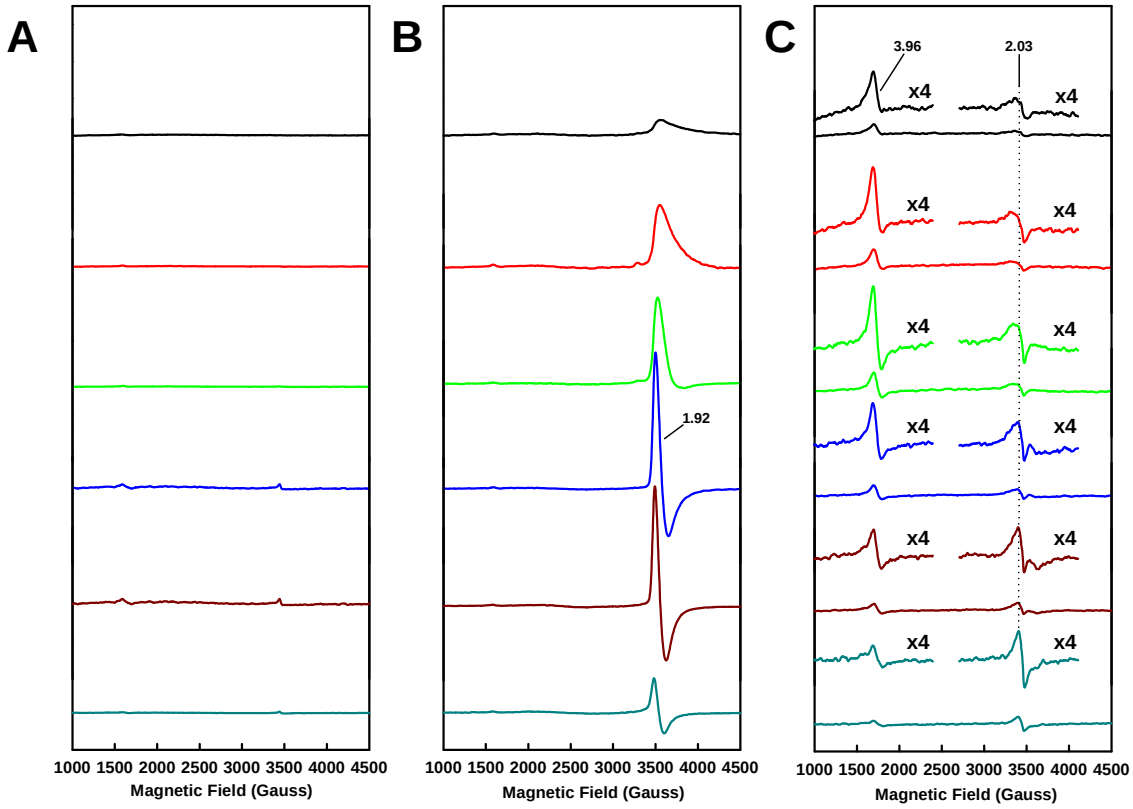

Figure S2. Temperature-dependency of EPR spectra of Δ*nifB* NifEN (A), NifEN^{Precursor} (B) and NifEN$^{\text{“FeMoco”(20 mM)}}$ (C) in IDS-oxidized states. The precursor-free Δ*nifB* NifEN (A) is EPR-silent upon IDS oxidation; whereas NifEN^{Precursor} (B) displays a precursor-specific signal at g = 1.92.[1] In the case of NifEN$^{\text{“FeMoco”(20 mM)}}$ (C), the features at g = 3.96 and 2.03 remain upon IDS oxidation. These features of NifEN$^{\text{“FeMoco”(20 mM)}}$ are enlarged, and the g values are given.

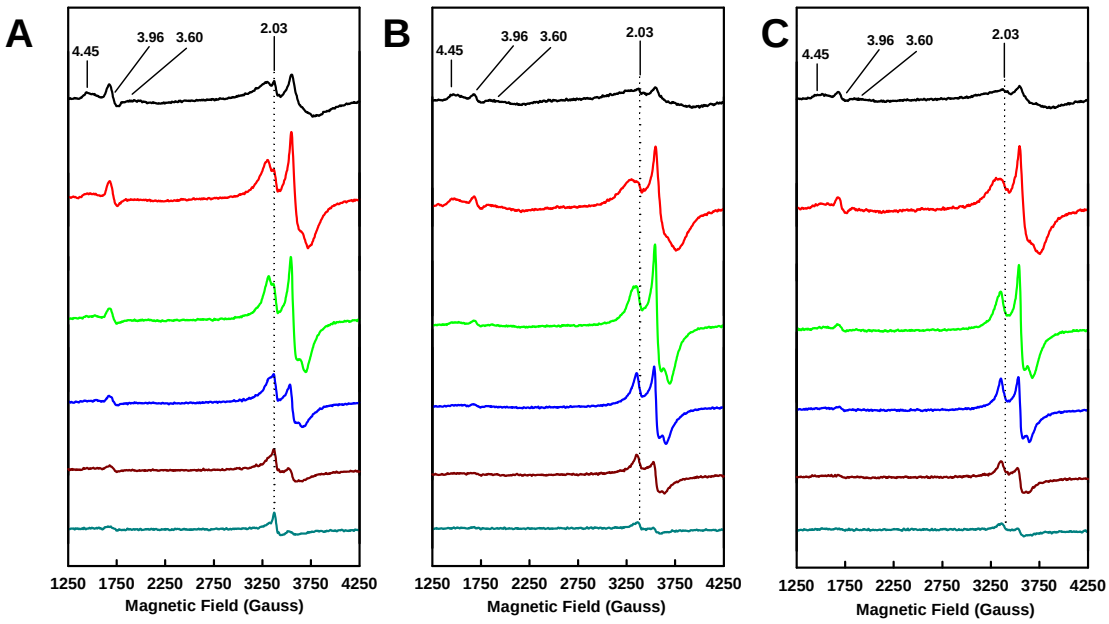

Figure S3. Temperature-dependency of EPR spectra of NifEN$^{\text{“FeMoco”(20 mM)}}$ (A), NifEN$^{\text{“FeMoco”[Ti(III) citrate]}}$ (B) and NifEN$^{\text{“FeMoco”(Fld_1)}}$ (C) in dithionite-reduced states. All three NifEN species display the characteristic features of “FeMoco”, including the S = 3/2 signal with g values of 4.45, 3.96 and 3.60, and the g = 2.03 feature in the S = 1/2 region.

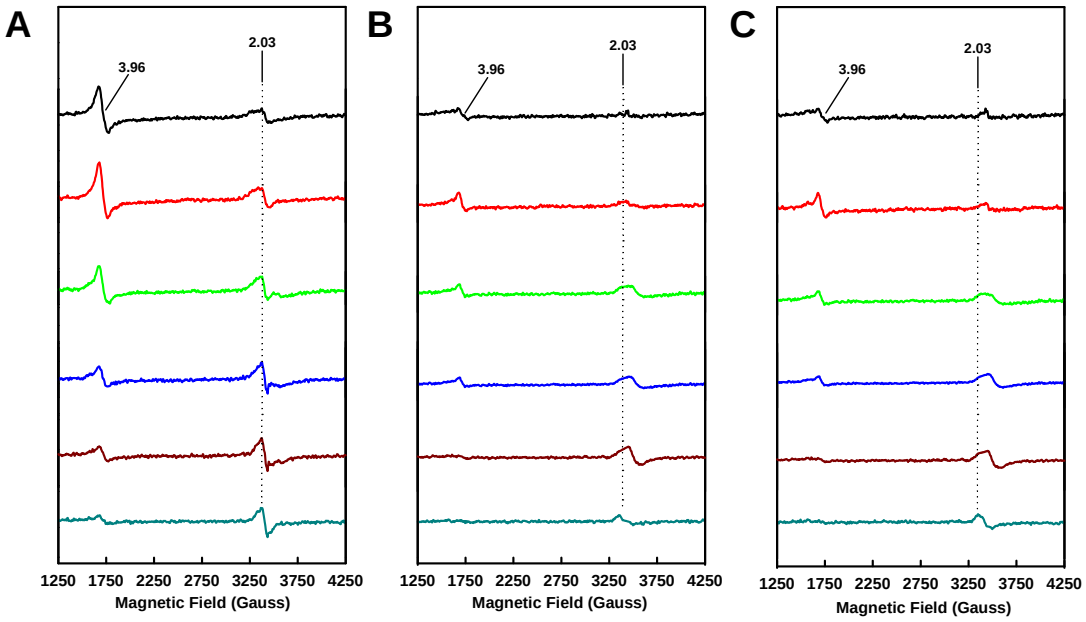

Figure S4. Temperature-dependency of EPR spectra of NifEN$^{\text{“FeMoco”(20 mM)}}$ (A), NifEN$^{\text{“FeMoco”[Ti(III) citrate]}}$ (B) and NifEN$^{\text{“FeMoco”(Fld_1)}}$ (C) in IDS-oxidized states. All three NifEN species display the characteristic features of “FeMoco”, including the g = 3.96 feature in the S = 3/2 region, and the g = 2.03 feature in the S = 1/2 region.

References

(1) Hu, Y.; Fay, A. W.; Ribbe, M. W. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 3236-3241.