SUPPORTING INFORMATION FOR

Spectroscopic characterization of mononitrosyl complexes in heme-nonheme diiron centers within the myoglobin scaffold (Fe_BMbs): relevance to denitrifying NO reductase†

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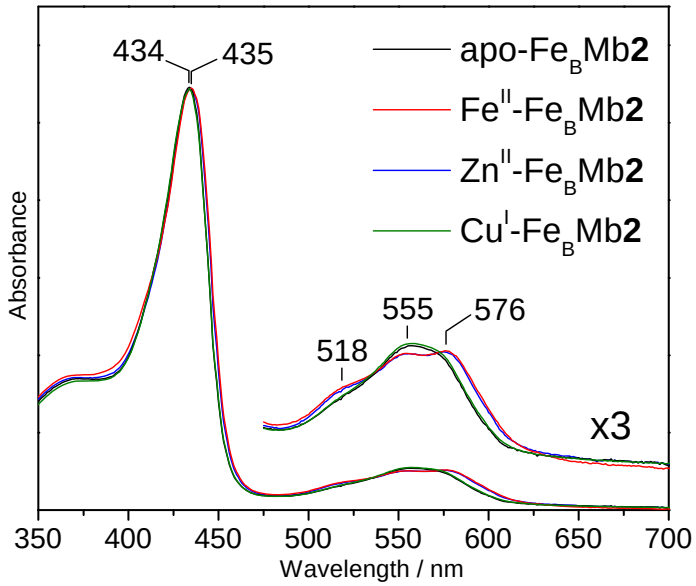

Figure S1. Room temperature UV-vis absorption spectra of reduced apo-, Fe^{II}-, Zn^{II}-, and Cu^{I}-Fe_BMb**2**.

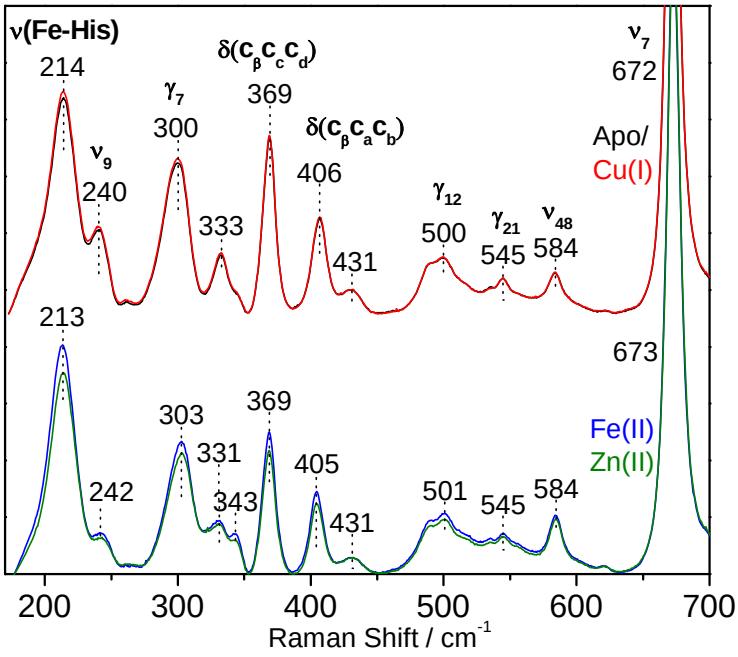

Figure S2. Room temperature RR spectra of reduced apo-, Fe^{II}-, Zn^{II}-, and Cu^{I}-Fe_BMb**2** obtained with 442-nm excitation.

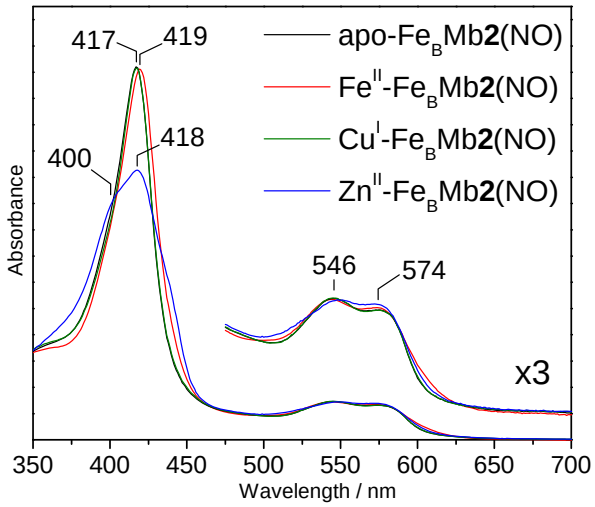

Figure S3. Room temperature UV-vis spectra of reduced apo-, Fe^{II}-, Zn^{II}-, and Cu^{I}-Fe_BMb**2** after addition of 1 equiv NO.

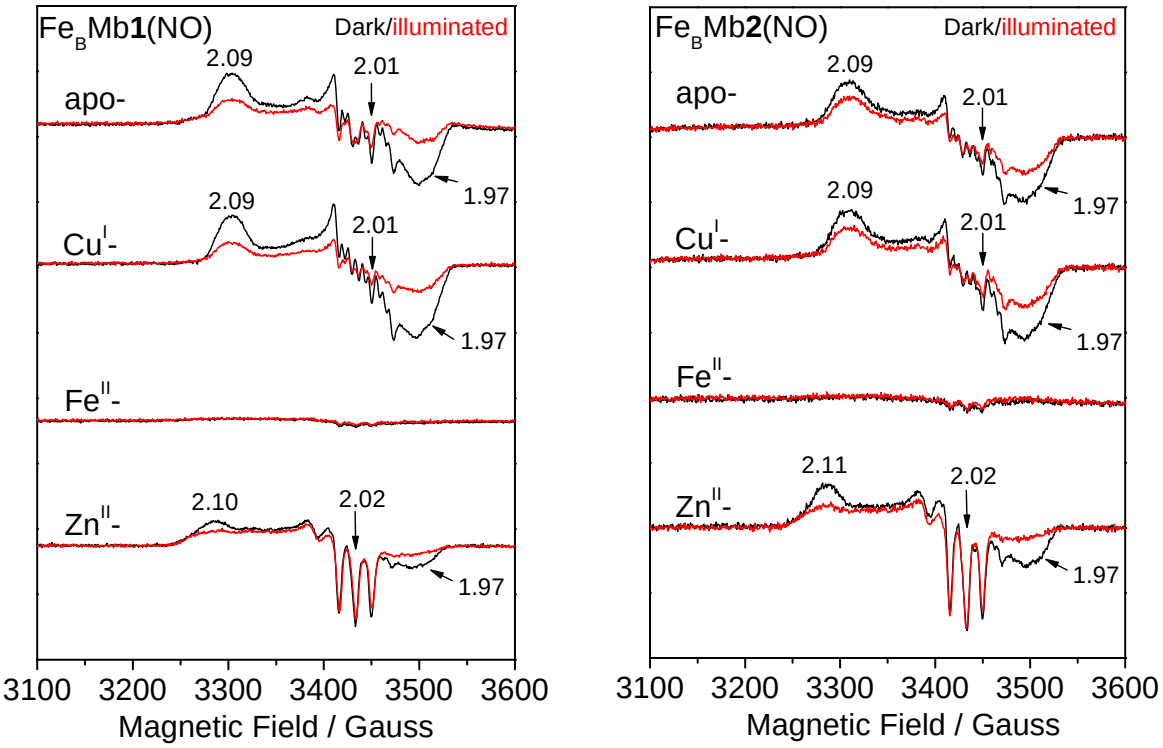

Figure S4. EPR spectra of apo-, Cu^{I}-, Fe^{II}-, and Zn^{II}-Fe_BMb**1**(NO) and equivalent Fe_BMb**2**(NO) complexes at 30 K. Comparing spectra obtained before (black) and after illumination (red) highlights resonances from the photolabile 6cLS heme $\{FeNO\}^7$ complexes. Conditions: microwave frequency, 9.7 GHz; microwave power, 0.25 mW; modulation frequency, 100 kHz; modulation amplitude, 4.0 G.

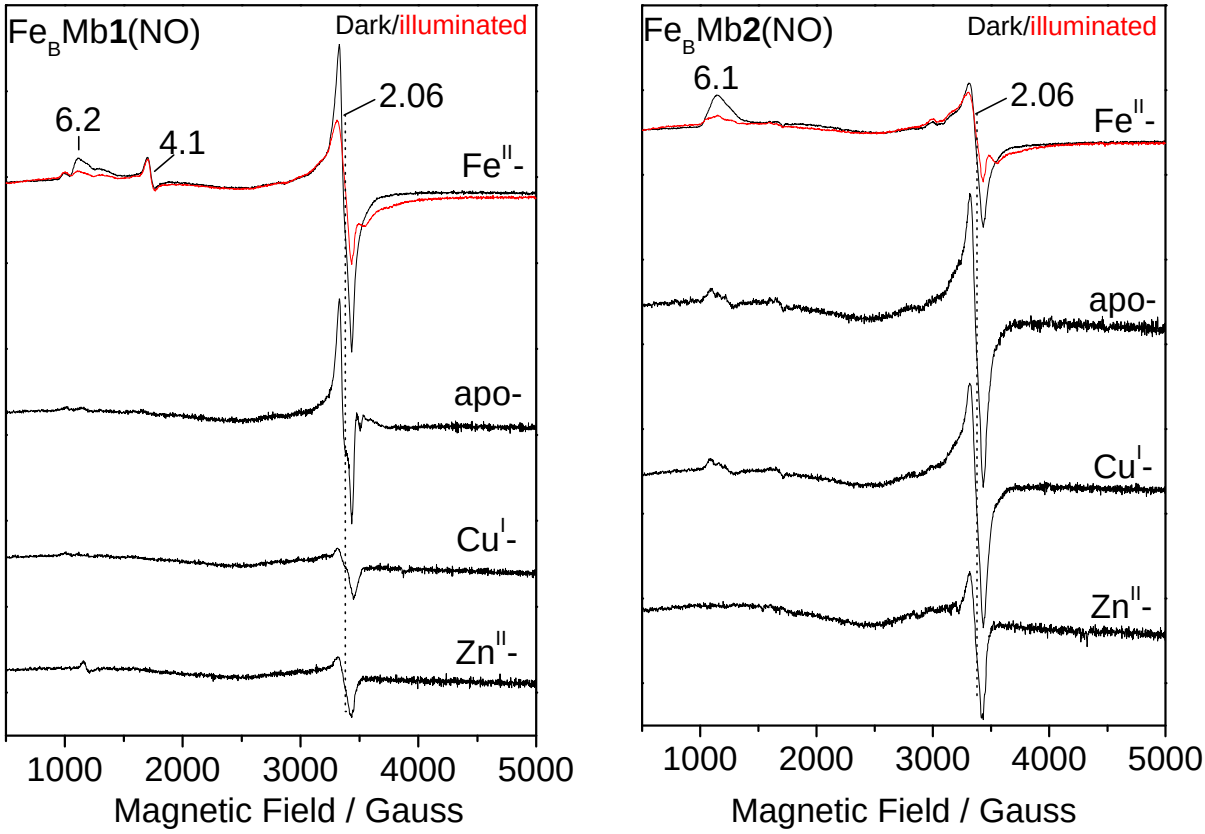

Figure S5. EPR spectra of Fe^{II}-Fe_BMb**1**(NO) and Fe^{II}-Fe_BMb**2**(NO) obtained at 4.2 K before (black) and after illumination (red). EPR spectra at 4.2 K of the apoproteins, Cu^{I}-, and Zn^{II}-loaded proteins are also shown for comparison. The high microwave power (20 mW) leads to saturating conditions for overlapping signals in the $g \sim 2$ region but it reveals non-saturating photolabile $g = 6.2$ and 6.1 resonances in Fe^{II}-Fe_BMb**1**(NO) and Fe^{II}-Fe_BMb**2**(NO), respectively. Prolonged incubation of the illuminated Fe^{II}-Fe_BMb**1**(NO) and Fe^{II}-Fe_BMb**2**(NO) samples in liquid nitrogen allows recovery of the photolabile $g = 6.2$ and 6.1 signals. These EPR signals are absent from other Fe_BMb(NO) complexes and Fe^{II}-Fe_BMb proteins (residual structured signals near $g \sim 6$ are assigned to minor Fe^{III} heme HS impurities and represent less than 1% of the overall heme content). Conditions: microwave frequency, 9.67 GHz; microwave power, 20 mW; modulation frequency, 100 kHz; modulation amplitude, 10.0 G.

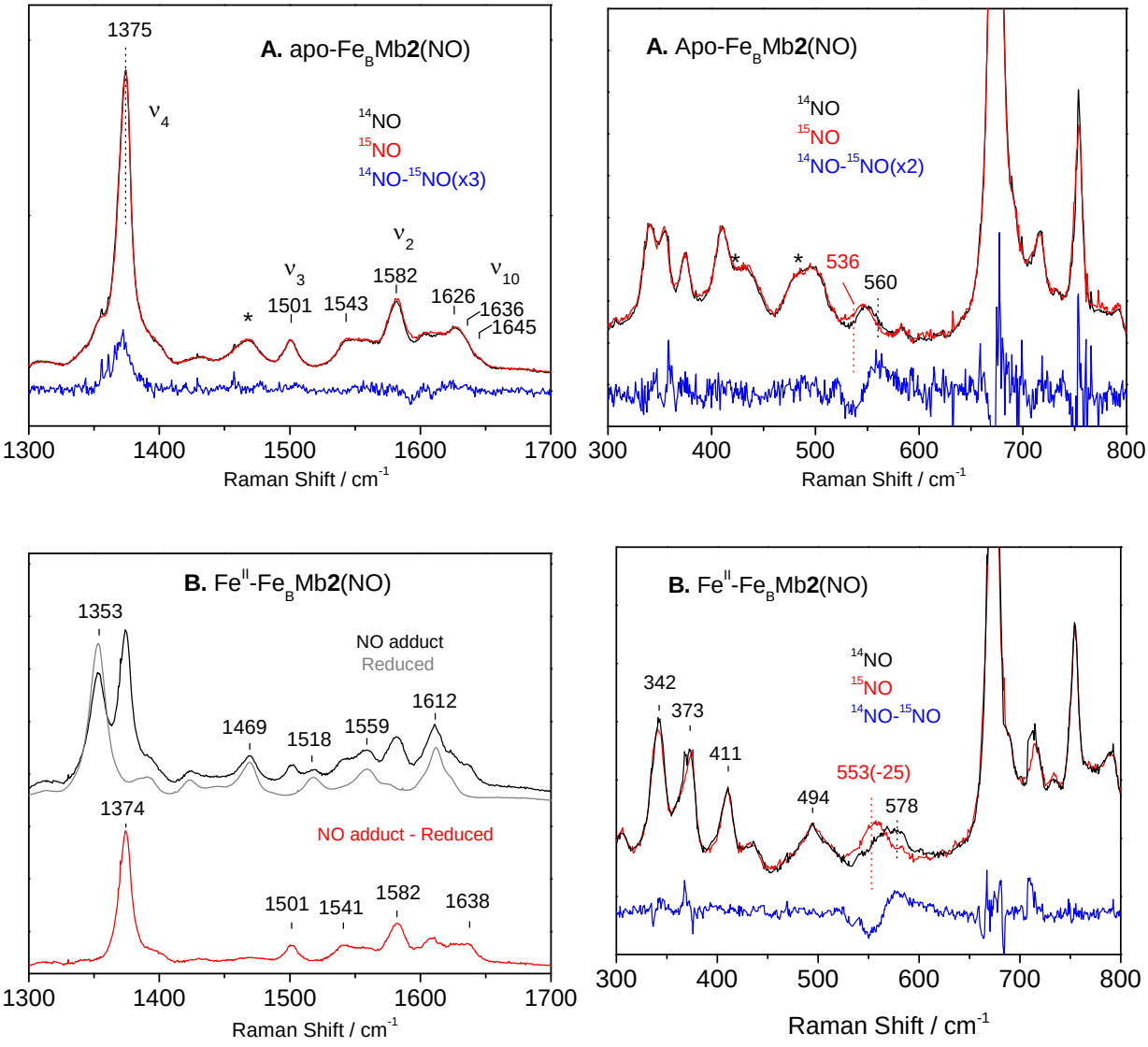

Figure S6. High- and low-frequency RR spectra of apo-Fe$_B$Mb**2**(NO) (A) and Fe^{II}-Fe$_B$Mb**2**(NO) (B) obtained with a 413-nm excitation at room temperature. Raman bands labeled with * are from glycerol.

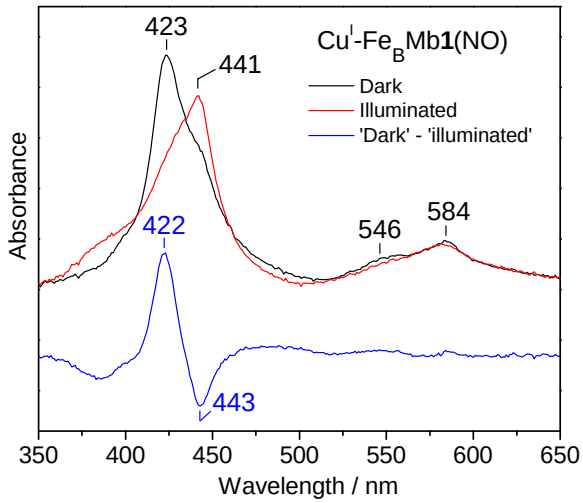

Figure S7. UV-vis spectra of Cu^{I}-Fe$_{B}$Mb**1**(NO) at 10K: dark (black), illuminated (red), and 'dark' minus 'illuminated' difference spectra (blue).

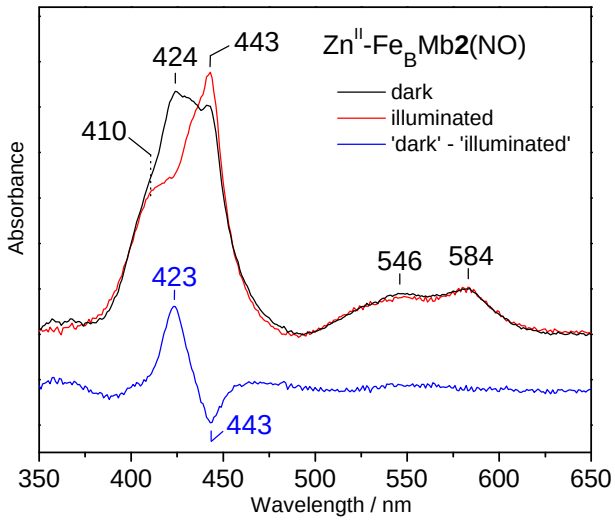

Figure S8. UV-vis spectra of Zn^{II}-Fe$_{B}$Mb**2**(NO) obtained at 10K: dark (black), illuminated (red), and 'dark' minus 'illuminated' difference spectra (blue).

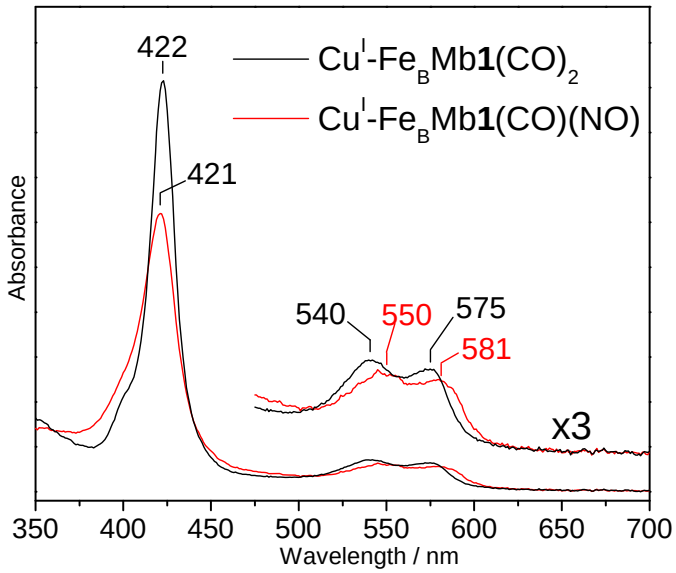

Figure S9. Room temperature UV-vis spectra of Cu^{I}-Fe$_{B}$Mb**1**(CO)$_2$ and Cu^{I}-Fe$_{B}$Mb**1**(CO)(NO).

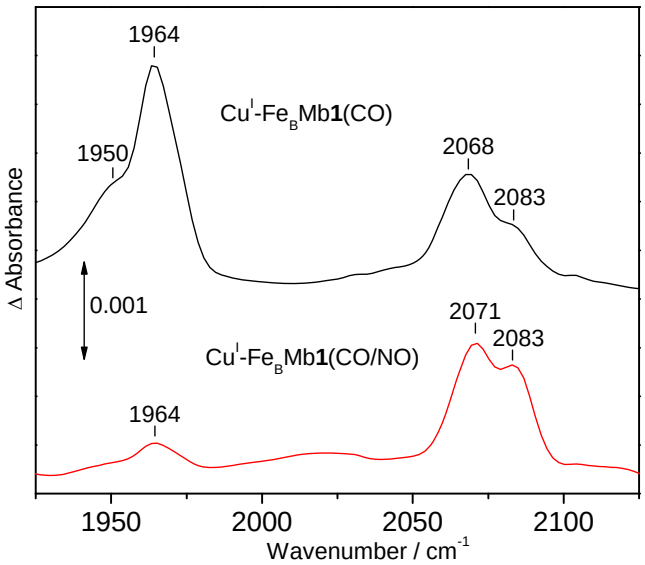

Figure S10. FTIR spectra of Cu^{I}-Fe$_{B}$Mb**1**(CO)$_2$ (black) and Cu^{I}-Fe$_{B}$Mb**1**(CO)(NO) (red) obtained in the dark at 10 K (the dark spectrum of Cu^{I}-Fe$_{B}$Mb**1(**NO) obtained at 10 K was used as background to isolate carbonyl stretching frequencies).

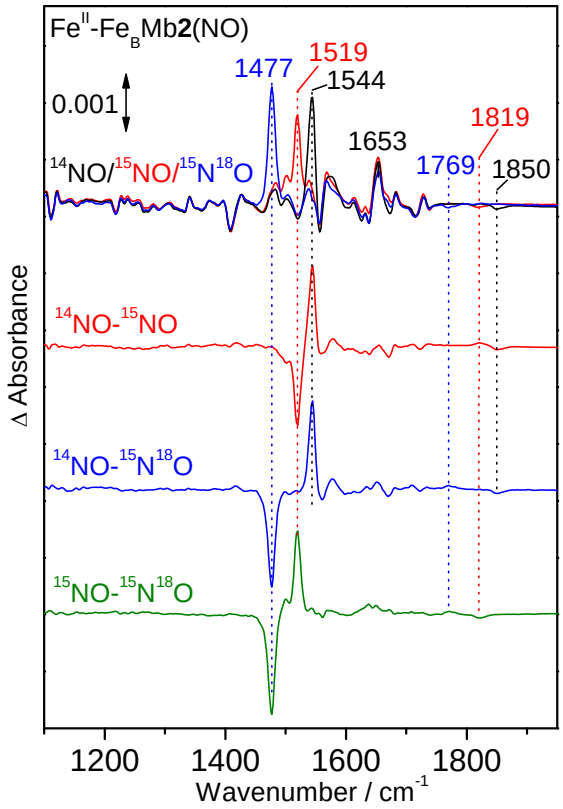

Figure S11. FTIR difference spectra ('dark' minus 'illuminated') of Fe^{II}-Fe_BMb**2**(NO) at 10 K.