Supplementary Information

Equilibrium between inactive ready Ni-SI_r and active Ni-SI_a states of [NiFe] hydrogenase studied by utilizing Ni-SI_r-to-Ni-SI_a photoactivation

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Experimental Section

Preparation of [NiFe] hydrogenase

[NiFe] hydrogenase (H_2ase) was isolated from sulfate reducing bacterium *Desulfovibrio vulgaris* Miyazaki F (*Dv*MF), and purified as described previously.[1] The concentration of [NiFe] H_2ase was adjusted with its absorption at 400 nm using its absorption coefficient ($\varepsilon = 47\ mM^{-1}cm^{-1}$).[2]

FT-IR measurements

[NiFe] H_2ase (concentration, ~2.0 mM) in 100 mM Tris-HCl buffer (pH 8.0, pH 8.5, and pD 8.0 at 274 K) was degassed with a vacuum line, purged with 1 bar of H_2, and incubated at 310 K for 5.5 h to obtain the H_2-activated sample.[3] The sample solution was further degassed with the vacuum line and purged with 1 bar of N_2.[4] The Ni-SI_r state was obtained by partial oxidation of the H_2-activated enzyme with an anaerobic addition of 5 equivalents of phenosafranine (Sigma-Aldrich) using a glove box (YSD-800L, UNICO, Tsukuba).[5] The sample solution was transferred anaerobically into an infrared cell with CaF_2 windows in the glove box. FT-IR spectra were measured before, during, and after light irradiation at 173–228 K with a FT-IR spectrometer (FT-IR 6100V, JASCO, Tokyo) equipped with an MCT detector. A cryostat system (CoolSpeK IR USP-203IR-A, Unisoku, Hirakata) was used to control the temperature of the cell. The light irradiation spectra were measured 2–5 min after light-irradiation was started. Light irradiation of the sample was performed at 514.5 nm with an Ar^+ laser (Model 2017, Spectra-Physics, Santa Clara). The laser power was adjusted to 3.3 W/cm^2 at the sample point. The corresponding buffer spectrum was collected as a reference spectrum and subtracted from the sample spectra. Spectral data were collected at 2- or 4-cm^{-1} resolution. The spectra of 2- and 4-cm^{-1} resolution required about 0.98 and 0.67 s, respectively, for each scan.

References

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2 T. Yagi, K. Kimura, H. Daidoji, F. Sakai and S. Tamura, *J. Biochem.*, 1976, **79**, 661-671.
3 H. Tai, K. Nishikawa, M. Suzuki, Y. Higuchi and S. Hirota, *Angew. Chem. Int. Ed.*, 2014, **53**, 13817-13820.
4 H. Tai, K. Nishikawa, S. Inoue, Y. Higuchi and S. Hirota, *J. Phys. Chem. B*, 2015, **119**, 13668-13674.
5 H. Tai, L. Xu, S. Inoue, K. Nishikawa, Y. Higuchi and S. Hirota, *Phys. Chem. Chem. Phys.*, 2016, **18**, 22025-22030.

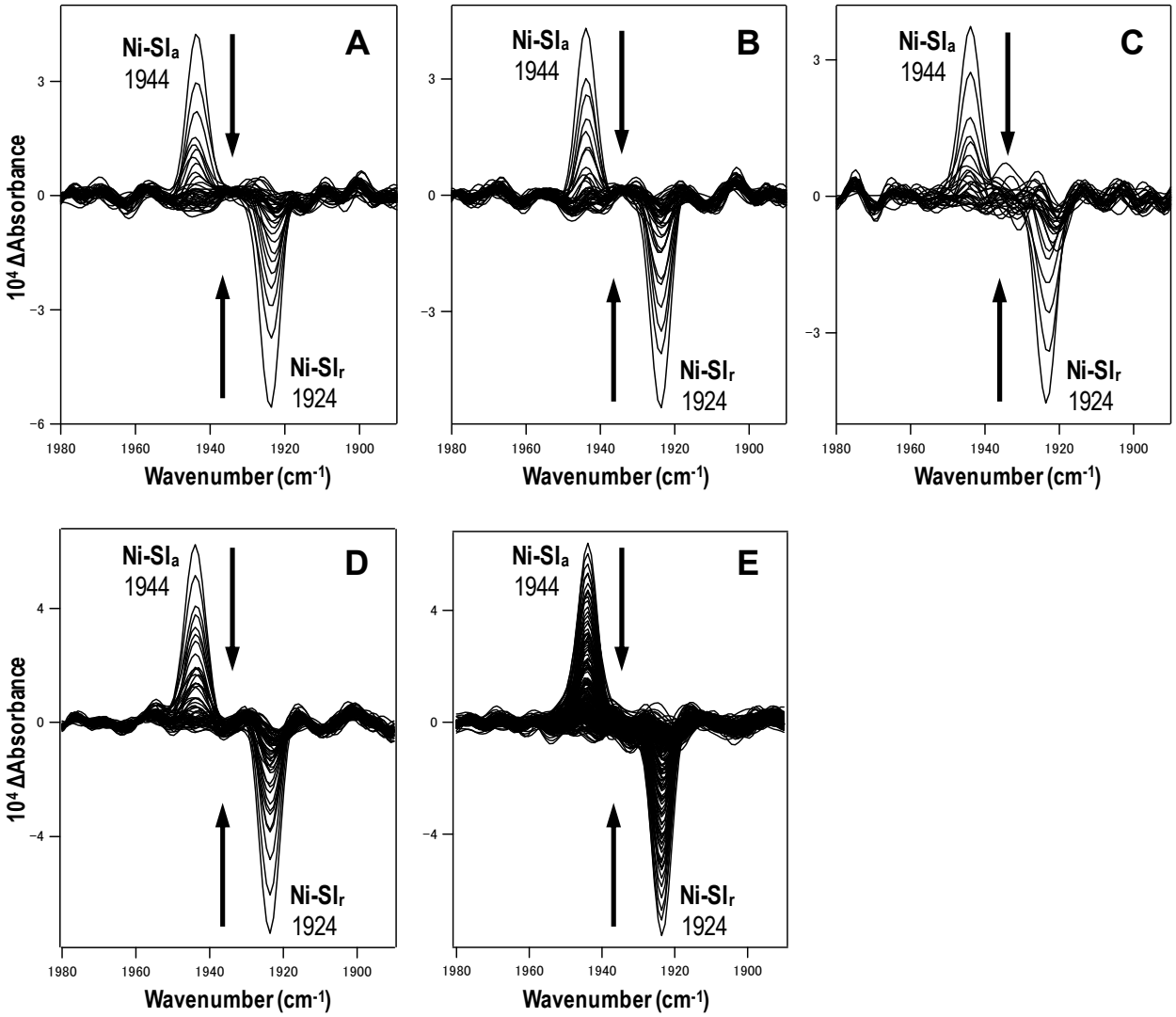

Fig. S1 Time-dependent after-minus-before difference FT-IR spectra of phenosafranine-oxidized *Dv*MF [NiFe] H2ase between the spectra after and before light irradiation at different temperatures at pH 8.0. The spectra were measured at (A) 203, (B) 198, (C) 193, (D) 188, and (E) 183 K, for (A) 0–0.25 to 10–10.25 min, average of 10 measurements, (B) 0–0.5 to 25–25.5 min, average of 4 measurements, (C) 0–1 to 64–65 min, average of 2 measurements, (D) 0–2 to 140–142 min, single measurement, and (E) 0–2 to 360–362 min, single measurement. The spectra were measured continuously without intervals. The spectra were collected at 4-cm^{-1} resolution.

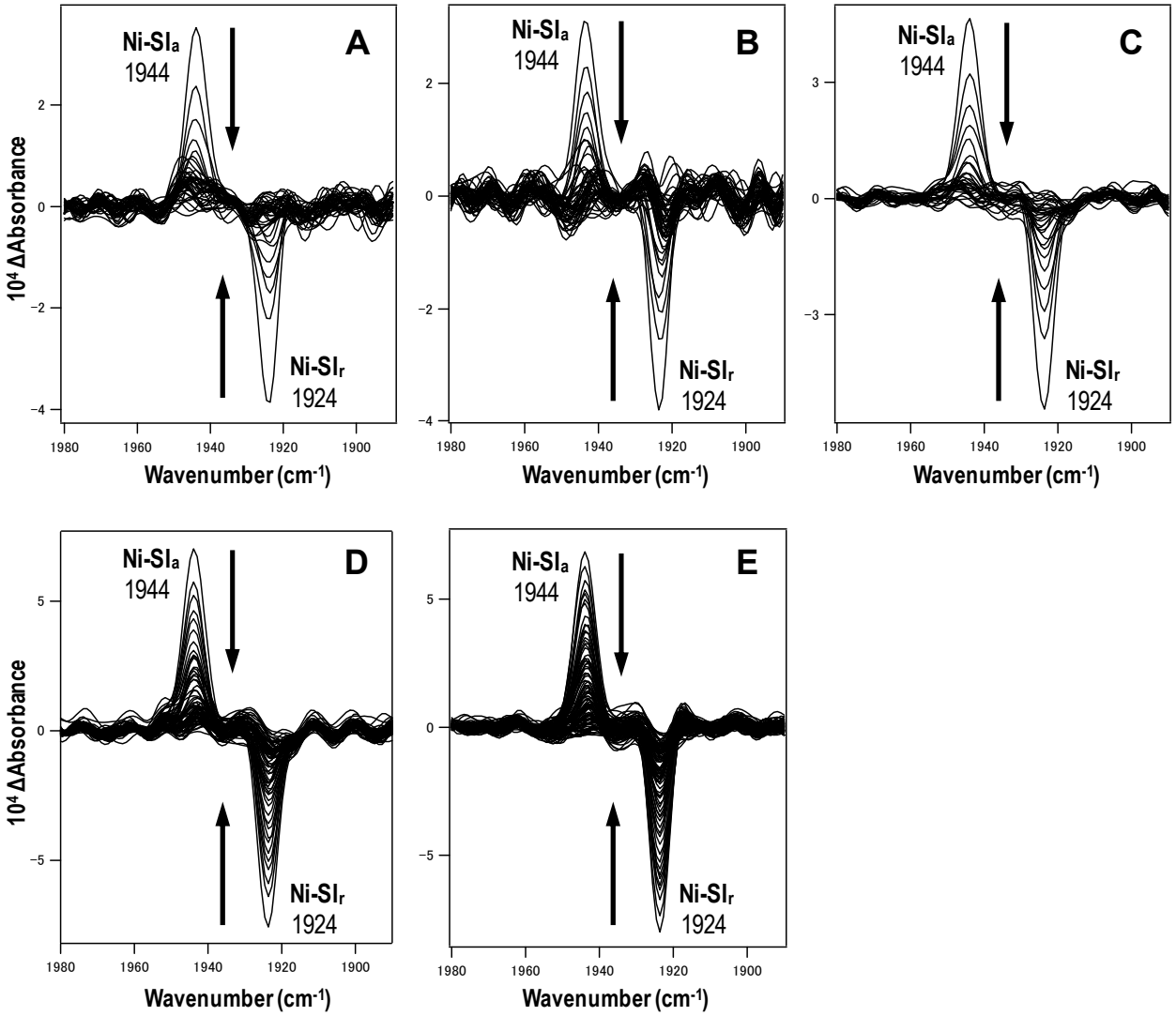

Fig. S2 Time-dependent after-minus-before difference FT-IR spectra of phenosafranine-oxidized *Dv*MF [NiFe] H_2ase between the spectra after and before light irradiation at different temperatures at pH 8.5. The spectra were measured at (A) 193, (B) 188, (C) 183, (D) 178, and (E) 173 K, for (A) 0–0.25 to 10–10.25 min, average of 10 measurements, (B) 0–0.5 to 25–25.5 min, average of 4 measurements, (C) 0–1 to 70–71 min, average of 2 measurements, (D) 0–2 to 150–152 min, single measurement, and (E) 0–2 to 360–362 min, single measurement. The spectra were measured continuously without intervals for (A–D) and 1-min intervals for (E). The spectra were not accumulated during the intervals. The spectra were collected at 4-cm^{-1} resolution.

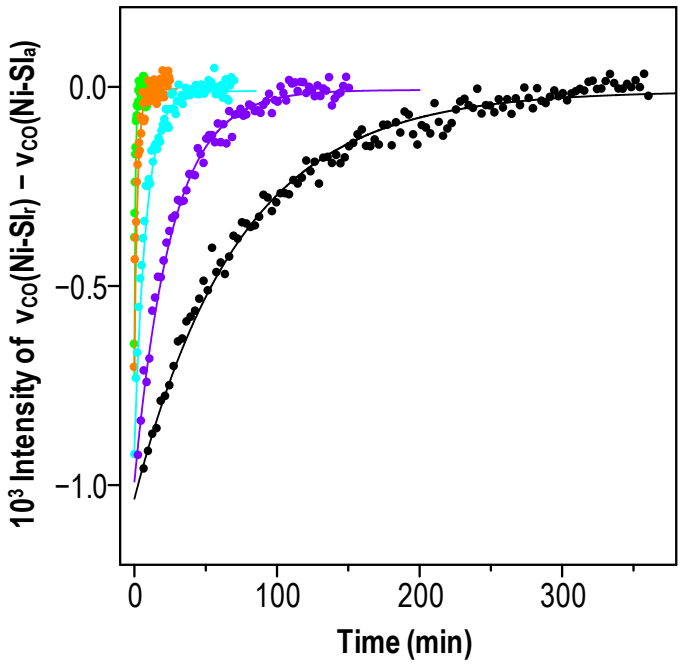

Fig. S3 Time dependence of the IR intensity difference at the νCO band frequencies during the conversion of the Ni-SIa state to Ni-SIr state for phenosafranine-oxidized *Dv*MF [NiFe] H2ase (νCO(Ni-SIr) minus νCO(Ni-SIa)) at 173 (black), 178 (purple), 183 (cyan), 188 (orange), and 193 K (green). The intensities were measured at pH 8.5.

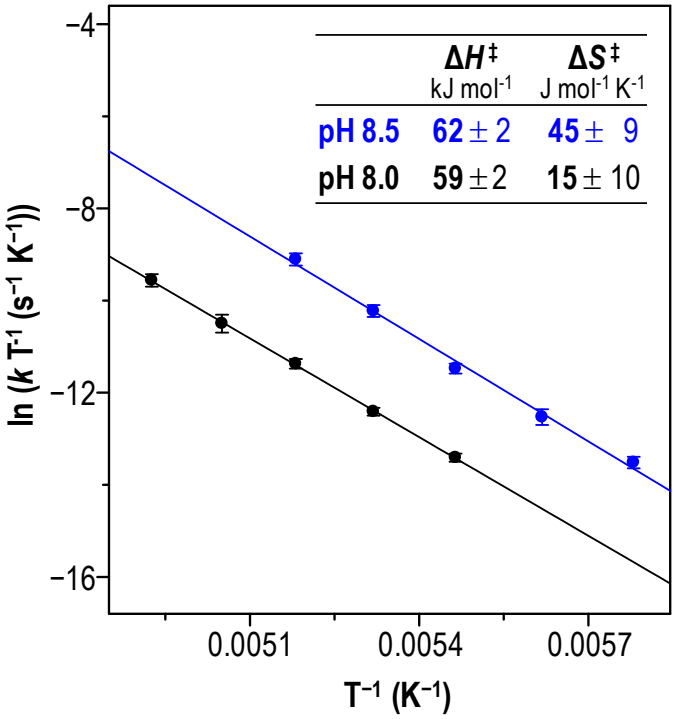

Fig. S4 Reciprocal temperature dependence of the rate constants for the conversion of the Ni-SI_a state to Ni-SI_r state of *Dv*MF [NiFe] H_2ase at pH 8.0 (black) and pH 8.5 (blue). The ln(k/T) values at different temperatures followed the Eyring equation, $\ln(k/T) = -\Delta H^{\ddagger}/(RT) + \Delta S^{\ddagger}/T + \ln(k_B/h)$, where T, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, R, k_B, and h represent the temperature, activation enthalpy, activation entropy, universal gas constant 8.3145 J K^{-1} mol^{-1}, Boltzmann constant 1.3807 J K^{-1}, and Planck constant 6.6261 J s, respectively. The obtained $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values are shown in the inset.

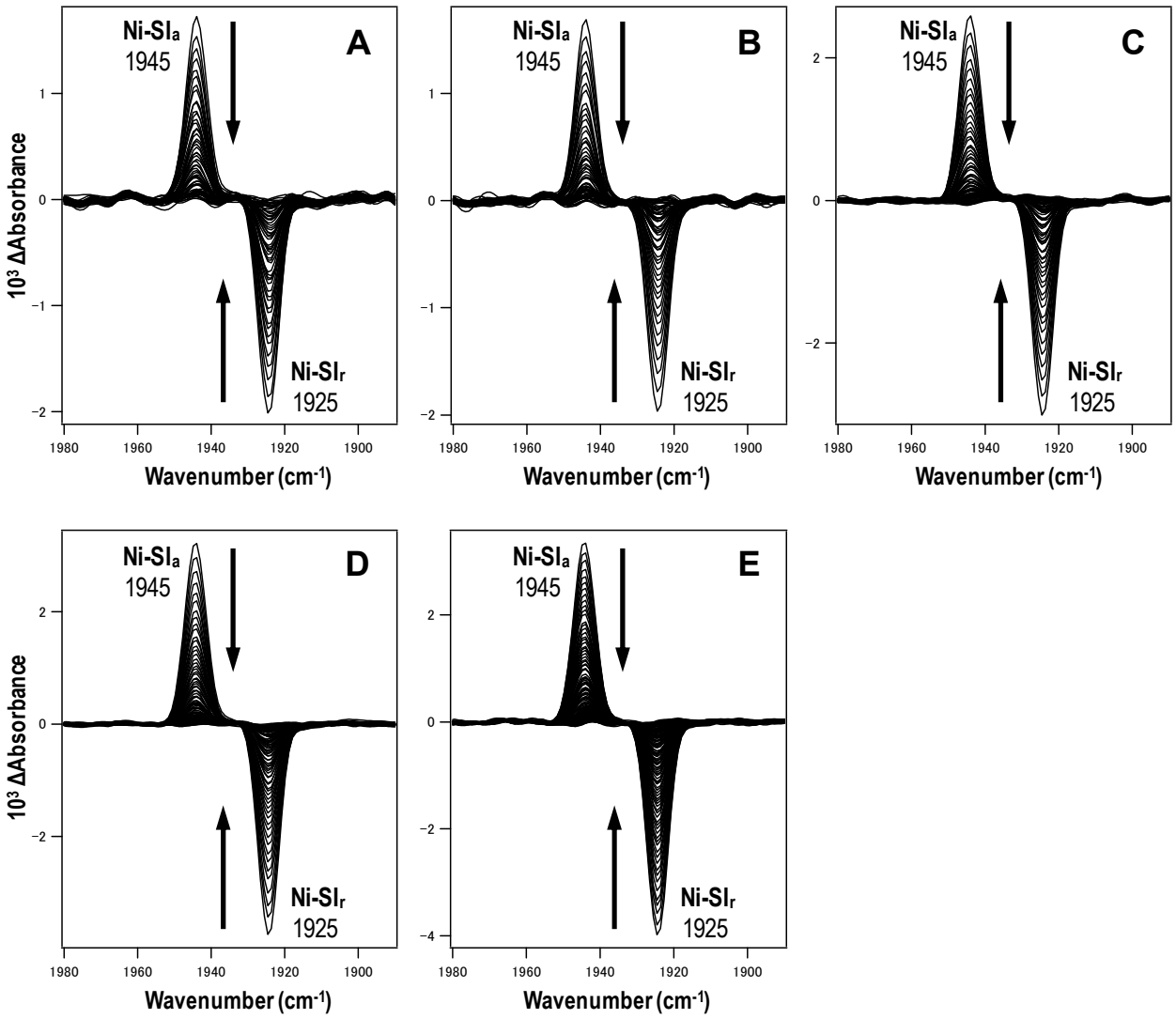

Fig. S5 Time-dependent after-minus-before difference FT-IR spectra of phenosafranine-oxidized *Dv*MF [NiFe] H_2ase between the spectra after and before light irradiation at different temperatures at pD 8.0. The spectra were measured at (A) 228, (B) 223, (C) 218, (D) 213, and (E) 208 K for (A) 0–0.16 to 12–12.16 min, average of 15 measurements, (B) 0–0.5 to 30–30.5 min, average of 4 measurements, (C) 0–1 to 80–81 min, average of 2 measurements, (D) 0–2 to 180–182 min, single measurement, and (E) 0–2 to 358–360 min, single measurement. The spectra were measured continuously without intervals for (A–D) and 1-min intervals for (E). The spectra were not accumulated during the intervals. The spectra were collected at 4-cm^{-1} resolution.

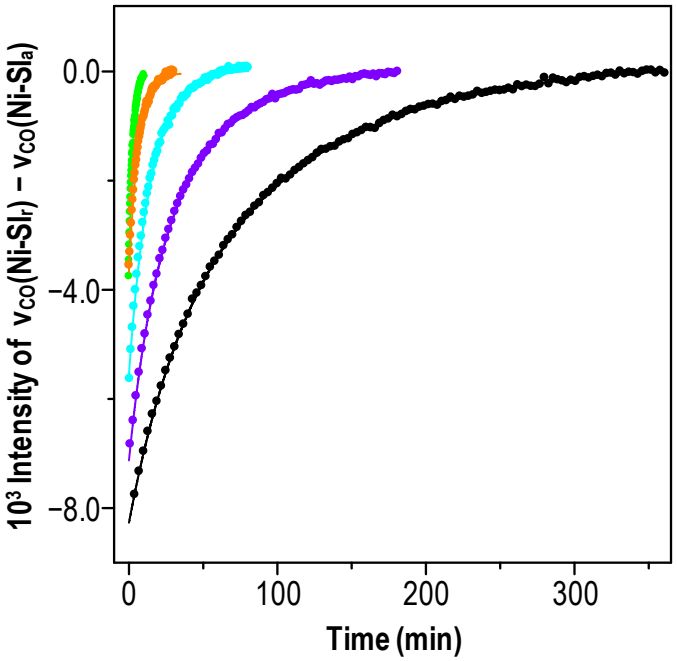

Fig. S6 Time dependence of the IR intensity difference at the ν_{CO} band frequencies during the conversion of the Ni-SI_a state to Ni-SI_r state for phenosafranine-oxidized *Dv*MF [NiFe] H_2ase (ν_{CO}(Ni-SI_r) minus ν_{CO}(Ni-SI_a)) at 208 (black), 213 (purple), 218 (cyan), 223 (orange), and 228 K (green). The intensities were measured at pD 8.0.