Supplementary Information

Photoactivation of the Ni-SI_r state to Ni-SI_a state in [NiFe] hydrogenase: FT-IR study on the light reactivity of the ready Ni-SI_r state and as-isolated enzyme revisited

Hulin Tai,[a,b] Liyang Xu,[a] Seiya Inoue,[c] Koji Nishikawa,[c] Yoshiki Higuchi,[b,c] and Shun Hirota[a,b,*]

[a]*Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama-cho, Ikoma-shi, Nara 630-0192, Japan,* [b]*CREST, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan, and* [c]*Graduate School of Life Science, University of Hyogo, 3-2-1 Koto kamigori-cho, Ako-gun, Hyogo 678-1297, Japan*

*E-mail: hirota@ms.naist.jp; Fax: +81-743-72-6119; Phone: +81-743-72-6110

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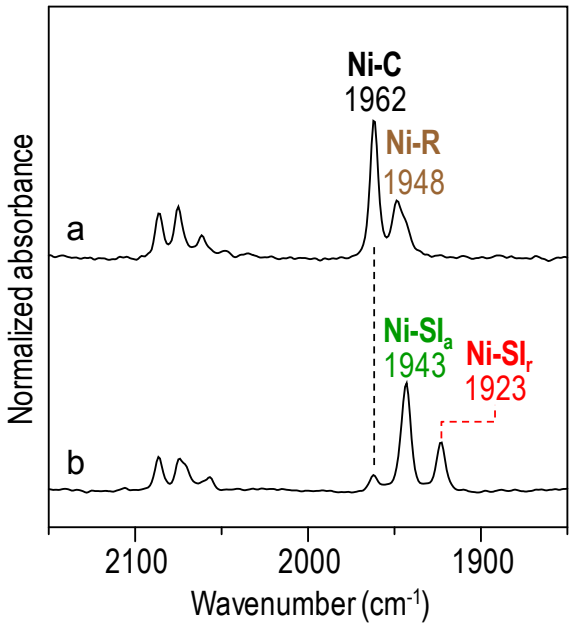

Fig. S1 FT-IR spectra of (a) H_2-activated and (b) phenosafranin-oxidized *Dv*MF [NiFe] hydrogenase under N_2 atmosphere at pH 7.4 and 298 K. Phenosafranin-oxidized [NiFe] hydrogenase was obtained by partial oxidation of the H_2-activated enzyme with an anaerobic addition of 5 equivalents of phenosafranin under N_2 atmosphere. The pH value was measured at 298 K. The ν_{CO} frequency of the Ni-SI_r state at 298 K was shifted to a lower frequency for about 1 cm^{-1} compared to that obtained at 178–238 K, although the frequency shift was smaller than the resolution (2 cm^{-1}).

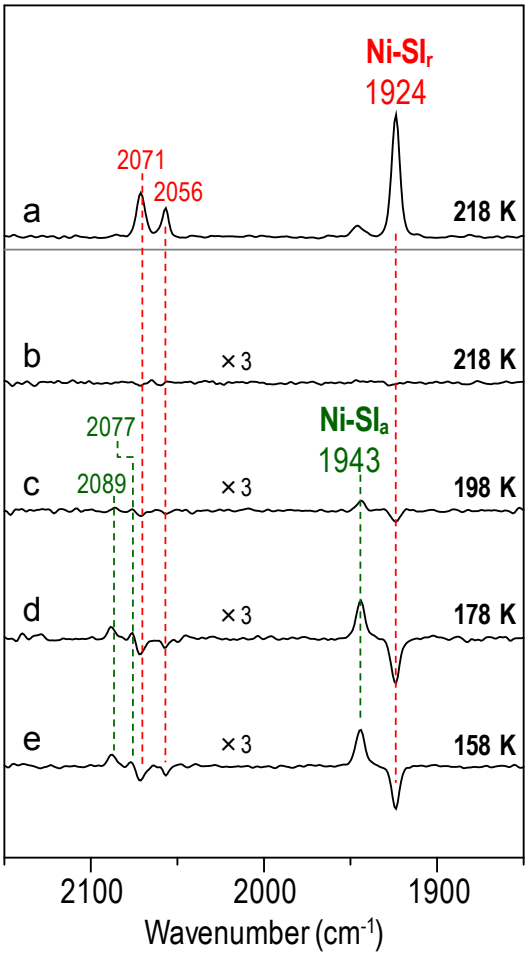

Fig. S2 FT-IR spectra of phenosafranin-oxidized *Dv*MF [NiFe] hydrogenase (a) before light irradiation at 218 K and (b–e) light-minus-before difference spectra between the spectra during and before light irradiation at 158–218 K under N_2 atmosphere at pH 9.6. Phenosafranin-oxidized [NiFe] hydrogenase was obtained by partial oxidation of the H_2-activated enzyme with an anaerobic addition of 5 equivalents of phenosafranin under N_2 atmosphere. The laser power was adjusted to 2.5 W/cm^2 at the sample point. The pH value was measured at 274 K.

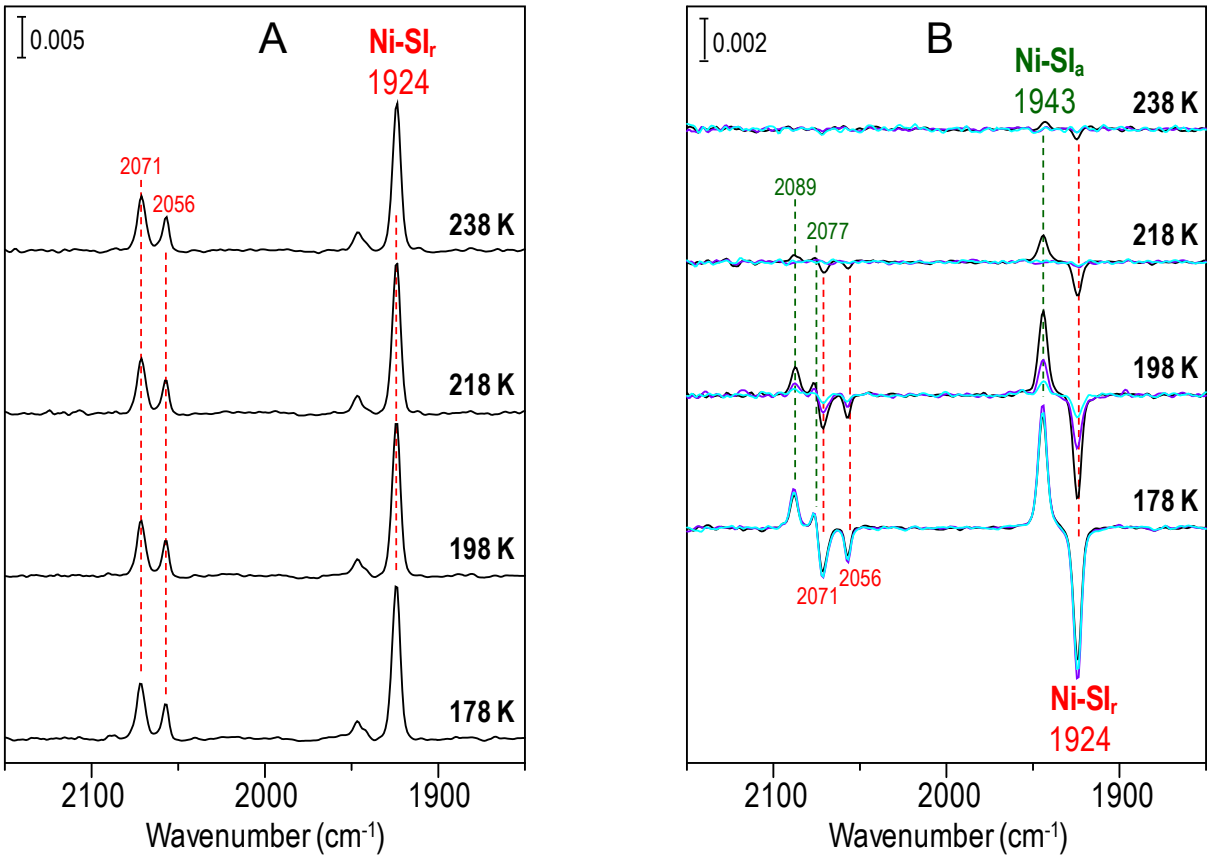

Fig. S3 FT-IR spectra of phenosafranin-oxidized *Dv*MF [NiFe] hydrogenase before light irradiation and their light-minus-before and after-minus-before difference spectra at different temperatures (178, 198, 218, and 238 K) under N_2 atmosphere at pH 8.0. (A) FT-IR spectra before light irradiation and (B) light-minus-before difference spectra between the spectra during and before light irradiation (black), and after-minus-before difference spectra between after and before light irradiation (magenta and cyan) are shown. The "after" spectra were measured 5–22 min (magenta) and 30–47 min (cyan) after light irradiation was stopped. Phenosafranin-oxidized [NiFe] hydrogenase was obtained by partial oxidation of the H_2-activated enzyme with an anaerobic addition of 5 equivalents of phenosafranin under N_2 atmosphere. The laser power was adjusted to 2.5 W/cm^2 at the sample point. The pH value was measured at 274 K.

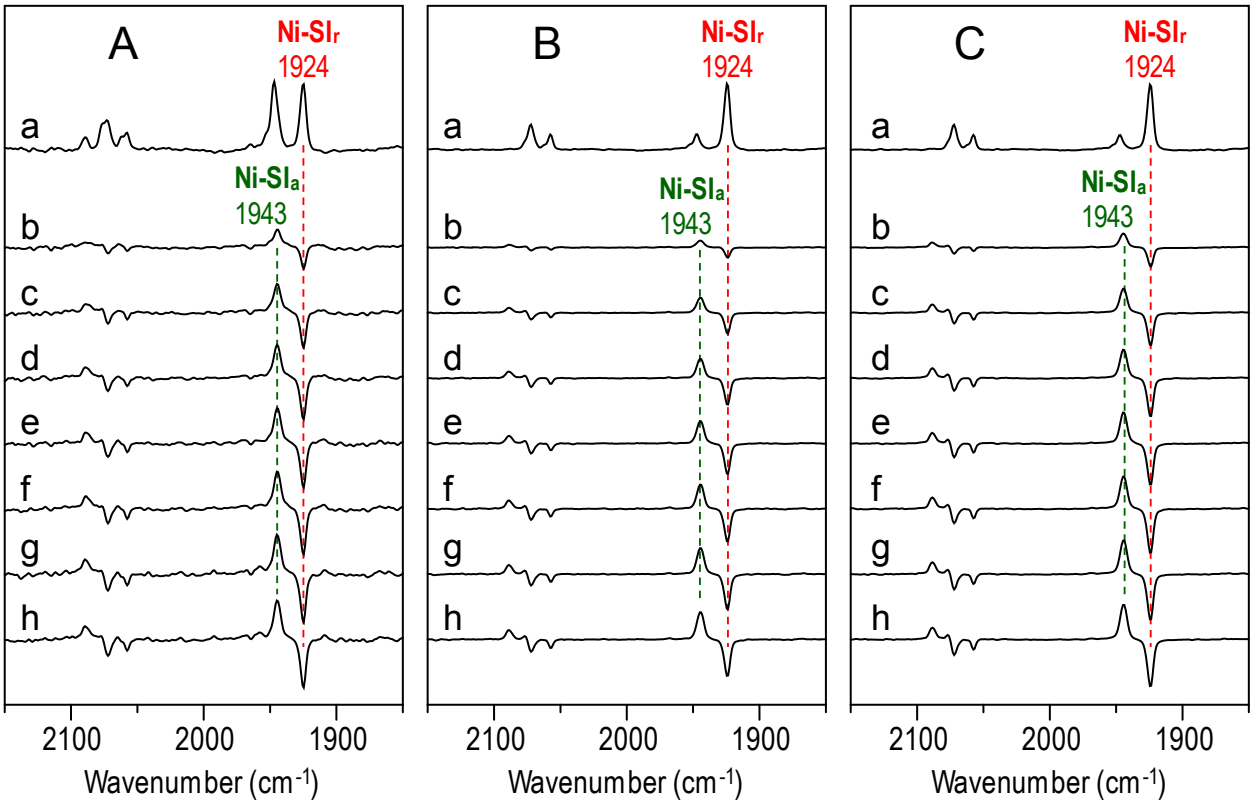

Fig. S4 FT-IR spectra of phenosafranin-oxidized *Dv*MF [NiFe] hydrogenase before light irradiation and their light-minus-before difference spectra under N_2 atmosphere at 103 K with different irradiation time and light intensity at (A) pH 7.0 and (B,C) pH 8.0. (a) FT-IR spectra before light irradiation and (b-h) light-minus-before difference spectra between the spectra during and before light irradiation are shown. The laser power was adjusted to (A,C) 3.3 and (B) 1.3 W/cm^2 at the sample point. The light-irradiation spectra were measured (b) 3–20, (c) 30–47, (d) 60–57, (e) 90–107, (f) 120–137, (g) 150–167, and (h) 180–197 min after light irradiation was started. Phenosafranin-oxidized [NiFe] hydrogenase was obtained by partial oxidation of the H_2-activated enzyme with an anaerobic addition of 5 equivalents of phenosafranin under N_2 atmosphere. The pH value was measured at 274 K.

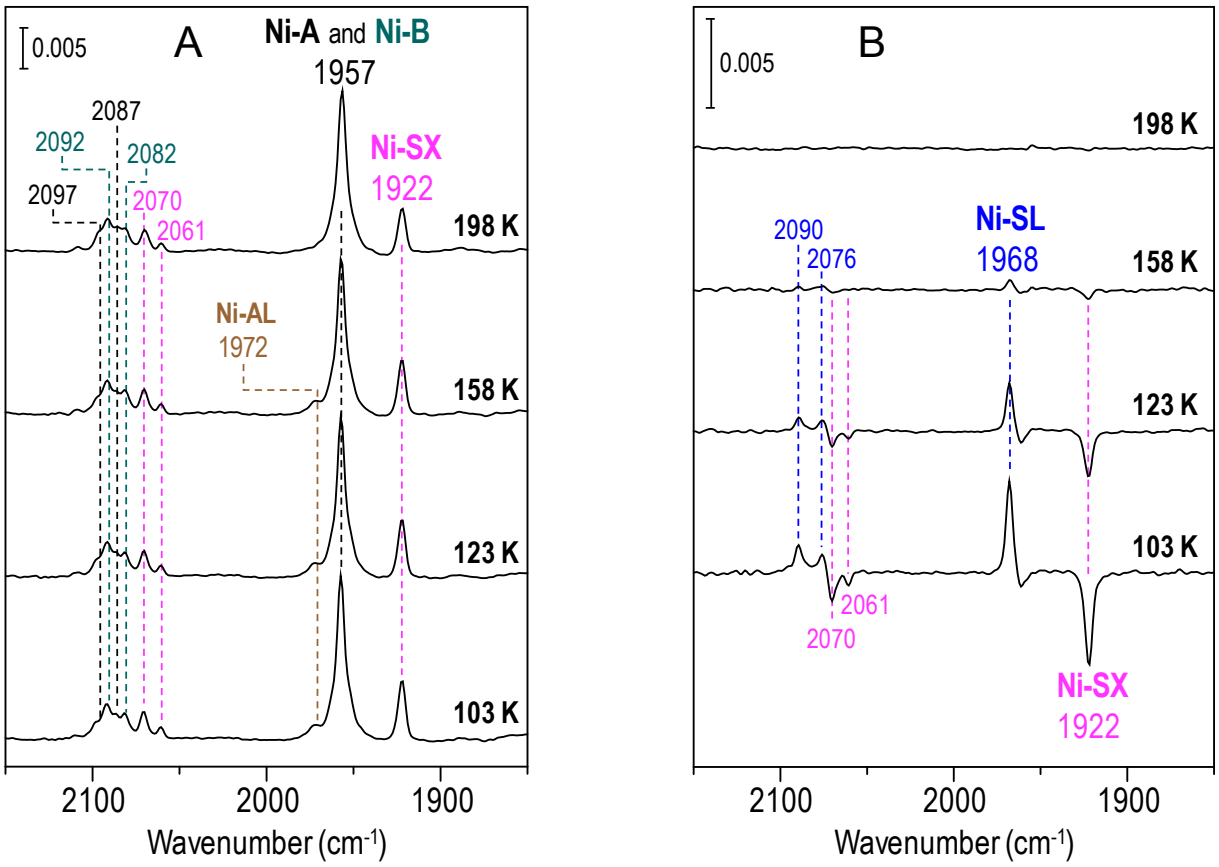

Fig. S5 FT-IR spectra of as-isolated *Dv*MF [NiFe] hydrogenase before light irradiation and their light-minus-after difference spectra at different temperatures (103, 123, 158, and 198 K) under N_2 atmosphere at pH 8.0. (A) FT-IR spectra before light irradiation and (B) light-minus-after difference spectra between the spectra during and after light irradiation. The "after" spectra were measured 5–22 min after light irradiation was stopped. The laser power was adjusted to 2.5 W/cm^2 at the sample point. The pH value was measured at 274 K.

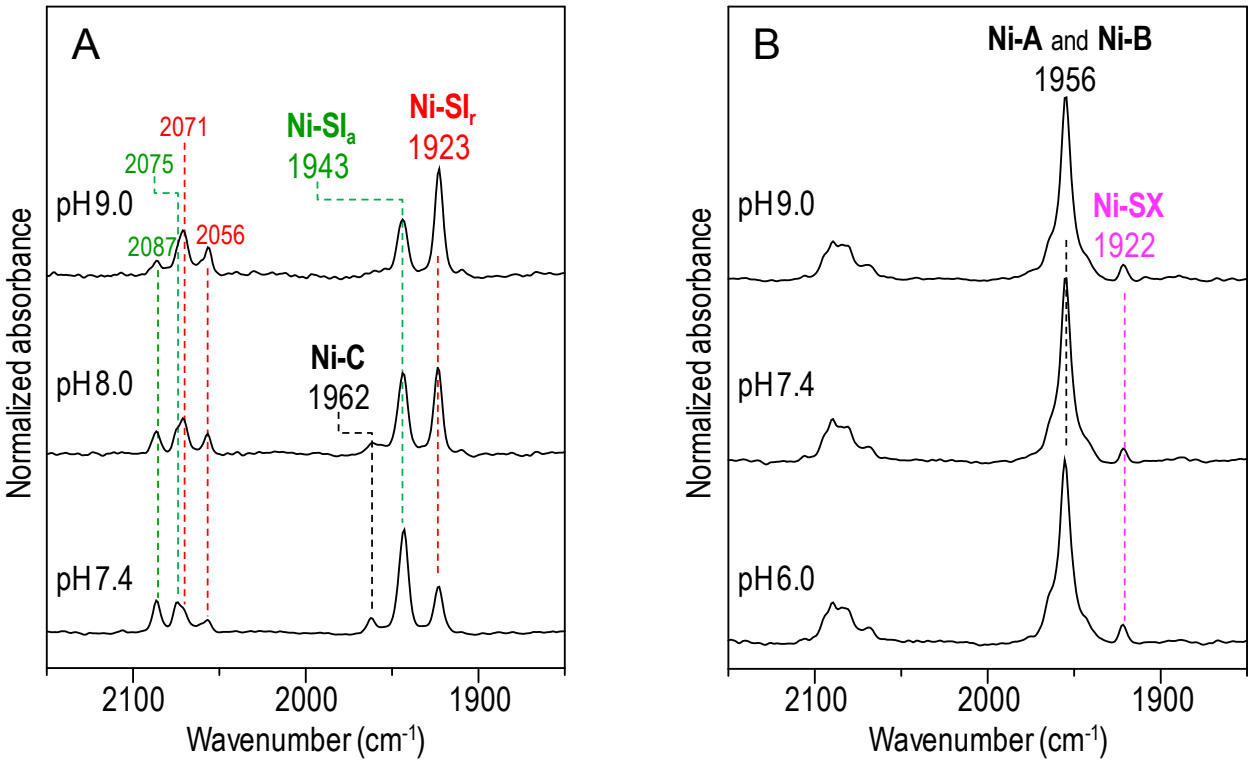

Fig. S6 FT-IR spectra of *Dv*MF [NiFe] hydrogenase under N_2 atmosphere at different pH values (pH 6.0, 7.4, 8.0, and 9.0) at 298 K: (A) Phenosafranin-oxidized and (B) as-isolated [NiFe] hydrogenase. Phenosafranin-oxidized [NiFe] hydrogenase was obtained by partial oxidation of the H_2-activated enzyme with an anaerobic addition of 5 equivalents of phenosafranin under N_2 atmosphere. The pH value was measured at 298 K.

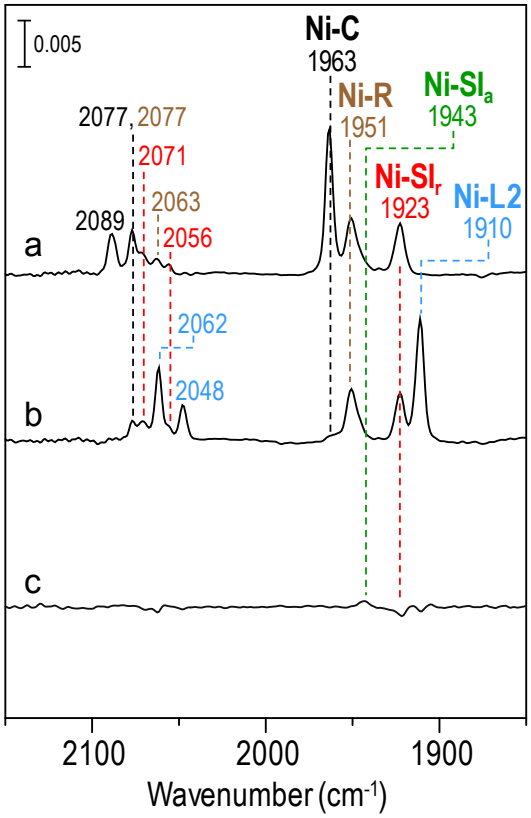

Fig. S7 FT-IR spectra of H_2-activated *Dv*MF [NiFe] hydrogenase before and after weak light irradiation and difference spectrum between the spectra during strong light irradiation and after week light irradiation under N_2 atmosphere at pH 8.0 and 103 K: (a) Before light irradiation, (b) after weak light irradiation, and (c) difference spectra between the spectra during strong light irradiation and after weak light irradiation. The weak and strong laser powers were adjusted to 0.5 and 2.5 W/cm^2, respectively, at the sample point. The "after" spectrum with weak light irradiation was measured 5–22 min after light irradiation was stopped. The pH value was measured at 274 K. No light-induced conversion of the Ni-SX state to Ni-SL state was observed in the difference spectrum.

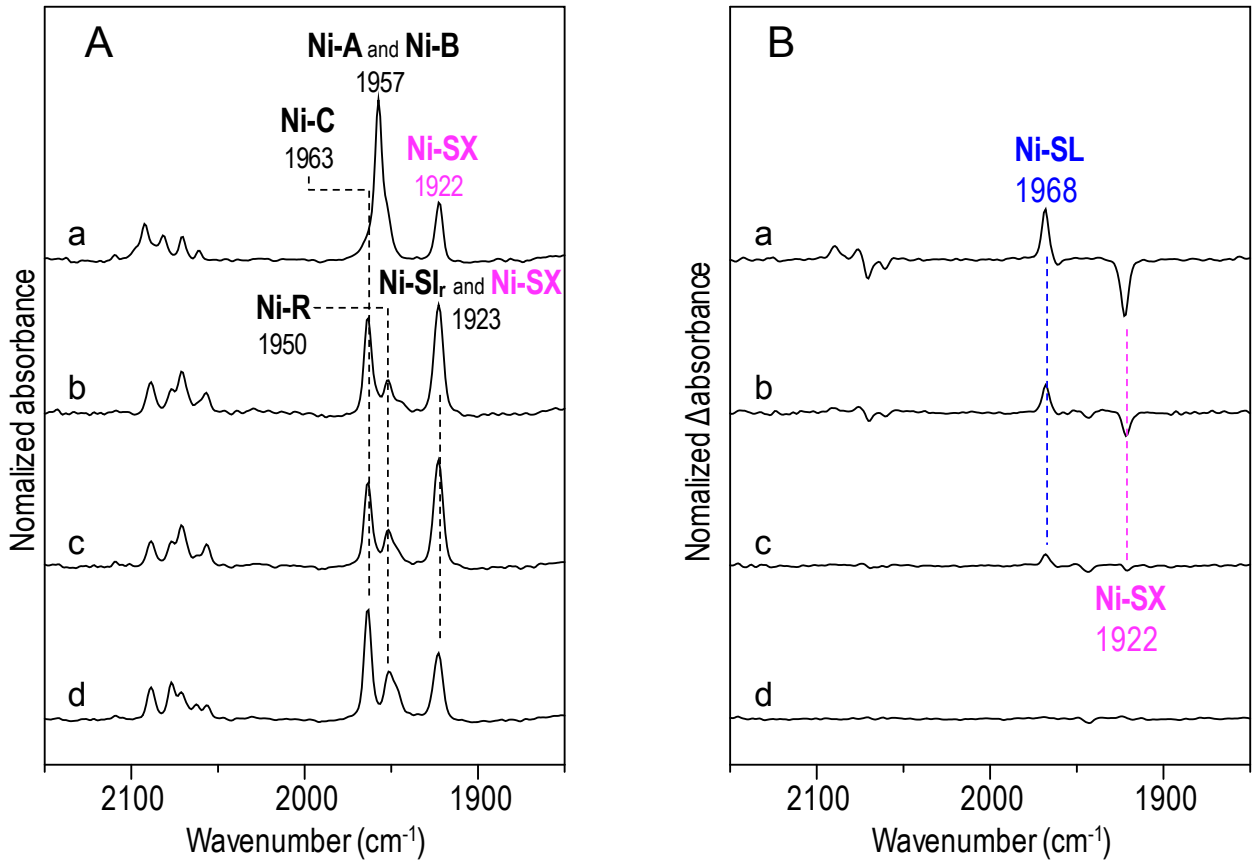

Fig. S8 FT-IR spectra of *Dv*MF [NiFe] hydrogenase (A) before light irradiation and (B) their light-minus-after difference spectra between the spectra during and after light irradiation under N_2 atmosphere at pH 8.0 and 103 K: (a) as-isolated and (b) 30 min, (c) 60 min, and (d) 120 min H_2-treated [NiFe] hydrogenase. To obtain the 30 min, 60 min, and 120 min H_2-treated [NiFe] hydrogenase, the as-isolated enzyme solution was degassed with a vacuum line, purged with 1 bar of H_2, and incubated at 310 K for (b) 30, (c) 60, and (d) 120 min, respectively. The "after" spectra were measured 30–47 min after light irradiation was stopped. The laser power was adjusted to 2.5 W/cm^2 at the sample point. The pH value was measured at 274 K.

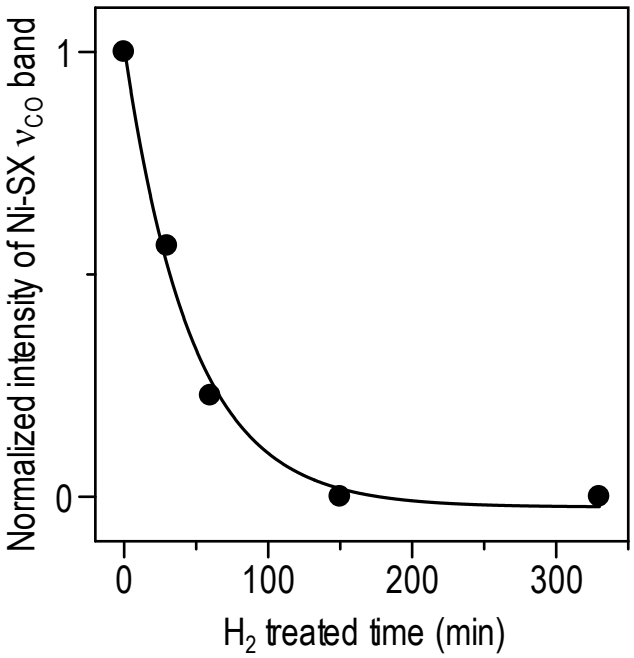

Fig. S9 H_2 activation kinetics of the Ni-SX state at 310 K. Intensities of the Ni-SX ν_{CO} band were calculated from Figures S7 and S8. The intensity of the ν_{CO} band decreased exponentially with a time constant of ~50 min.