Supplemental Materials

Structural Dynamics of the Heme Pocket and Intersubunit Coupling in the Dimeric Hemoglobin from *Scapharca inaequivalvis*

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S-1. Temporal Changes of the Band Frequencies and Intensities under Various Pumping Conditions

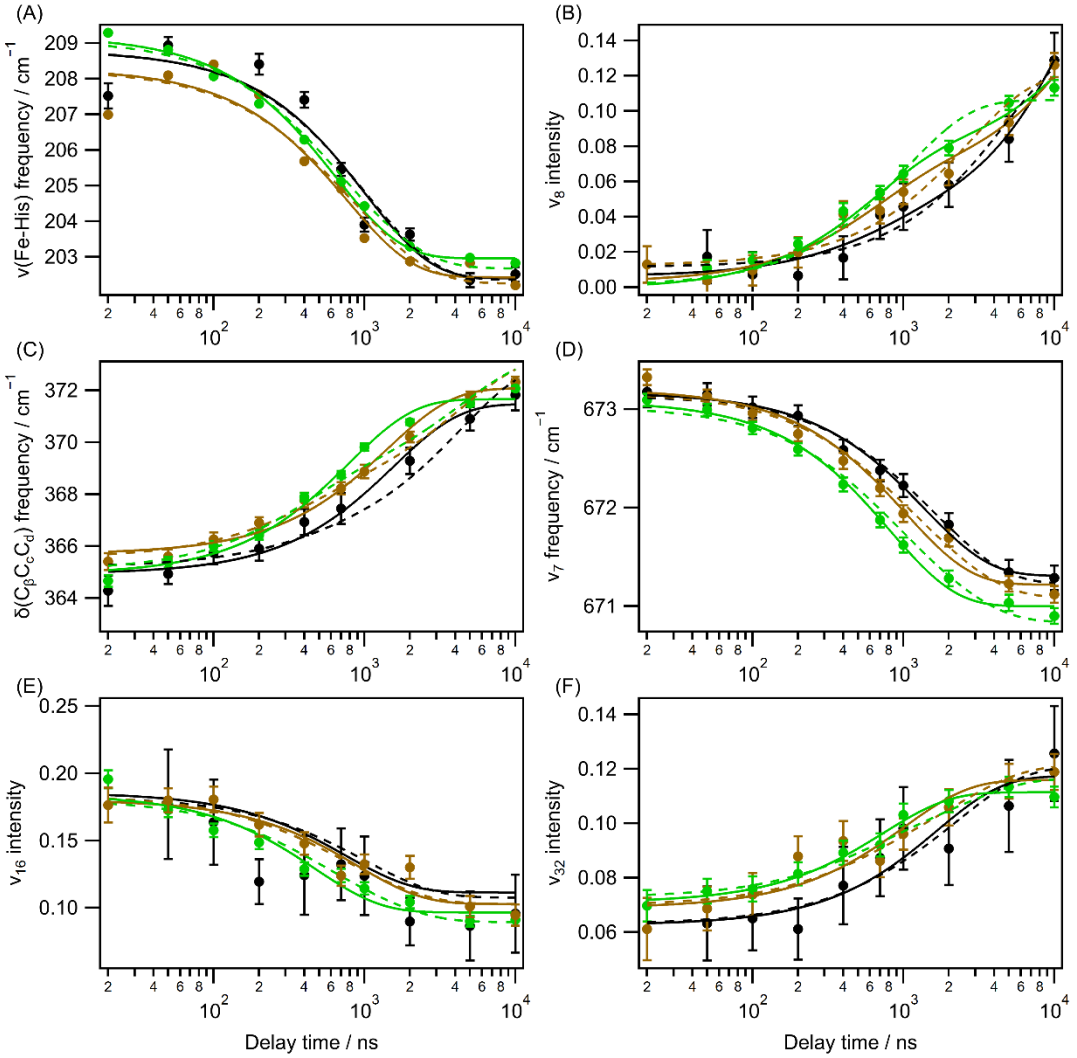

Figure S1. Temporal changes of the band frequencies and intensities under various pumping conditions. (A) ν(Fe–His) frequency, (B) ν_8 intensity, (C) $\delta(C_\beta C_c C_d)$ frequency, (D) ν_7 frequency, (E) ν_{16} intensity, and (F) ν_{32} intensity. Black, brown, and green markers show the frequencies and intensities for the pump energies of 7 μJ (dissociation fraction, 10.2%), 20 μJ (27.9%), and 30 μJ (38.3%), respectively. Solid lines are the best fits by a single exponential function. Broken lines show linear combinations of the contributions of singly and doubly dissociated HbI. The intensities of the ν_8, ν_{16}, and ν_{32} bands are shown as relative values to the intensity of the ν_7 band.

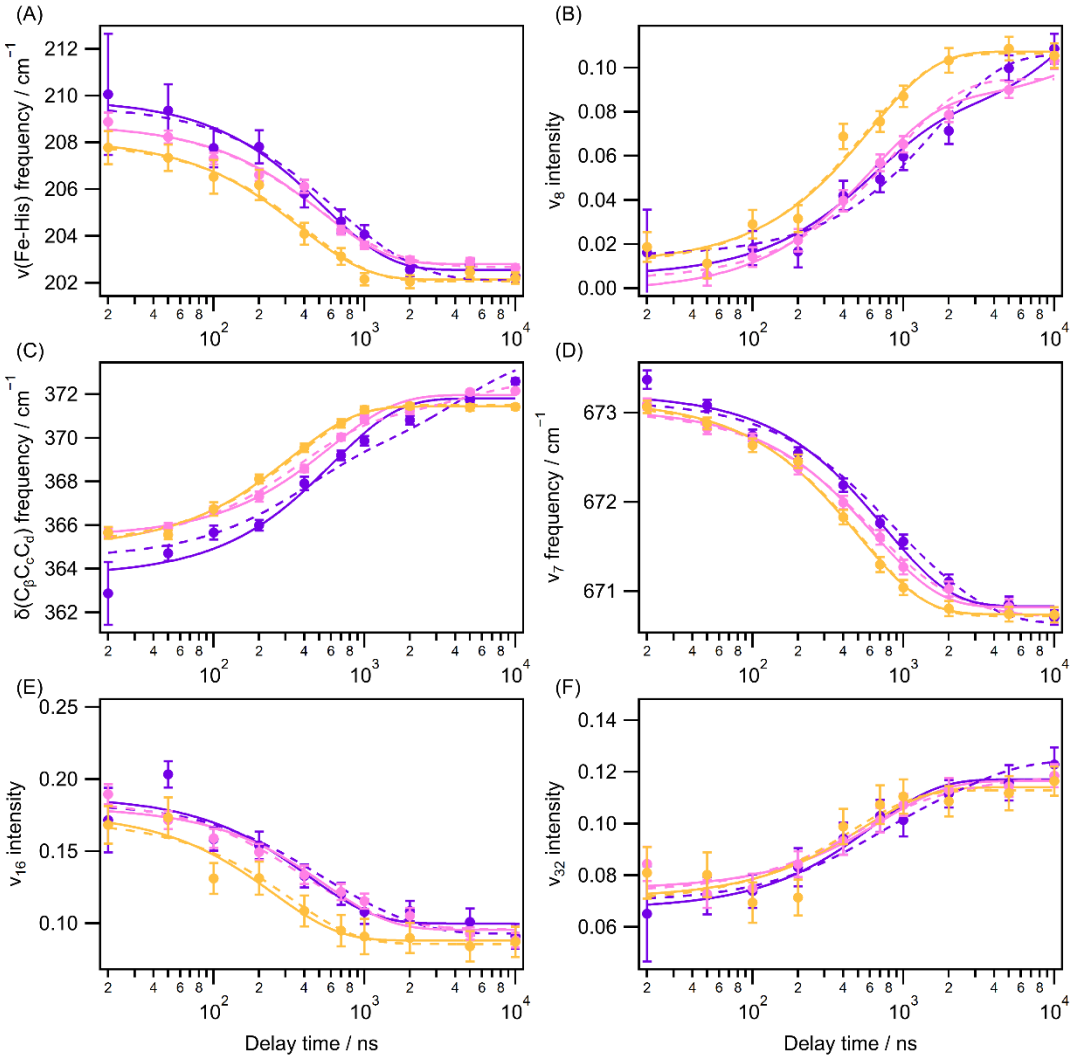

Figure S2. Temporal changes of the band frequencies and intensities under various pumping conditions. (A) ν(Fe–His) frequency, (B) ν_8 intensity, (C) δ($C_\beta C_c C_d$) frequency, (D) ν_7 frequency, (E) ν_{16} intensity, and (F) ν_{32} intensity. Purple, pink, and yellow markers show the frequencies and intensities for the pump energies of 40 μJ (dissociation fraction, 44.6%), 50 μJ (77.1%), and 350 μJ (100.0%), respectively. Solid lines are the best fits by a single exponential function. Broken lines show linear combinations of the contributions of singly and doubly dissociated HbI. The intensities of the ν_8, ν_{16}, and ν_{32} bands are shown as relative values to the intensity of the ν_7 band.

S-2. Procedures for Determining the Dissociation Fraction

We determined the dissociation fraction of HbI using the intensities of the ν_4 bands in the time-resolved resonance Raman (RR) spectra. The ν_4 band arises from the pyrrole half-ring stretching mode and is the most intense band in the RR spectra of heme. Two ν_4 bands were observed in the raw time-resolved RR spectrum at 10 μs (Figure S3A). The band at 1373 cm^{-1} is due to the unphotolyzed and CO-rebound forms. The band at 1356 cm^{-1} is attributed to the photodissociated species, since its frequency is close to that of the deoxy form. The bands at 1373 and 1356 cm^{-1} decreased and increased, respectively, as the energy of the pump pulse increased,[1] indicating that more photodissociated species were generated as the pump energy increased. The time-resolved spectra at 10 μs were fitted using a linear combination of the CO-bound and deoxy spectra to determine the fraction of the dissociated heme (dissociation fraction), f_{dis},

$$S_{\mathrm{fit}} = f_{\mathrm{dis}} S_{\mathrm{deoxy}} + (1 - f_{\mathrm{dis}}) S_{\mathrm{CO\text{-}bound}}. \quad (1)$$

An example of the fit is shown in Figure S3B.

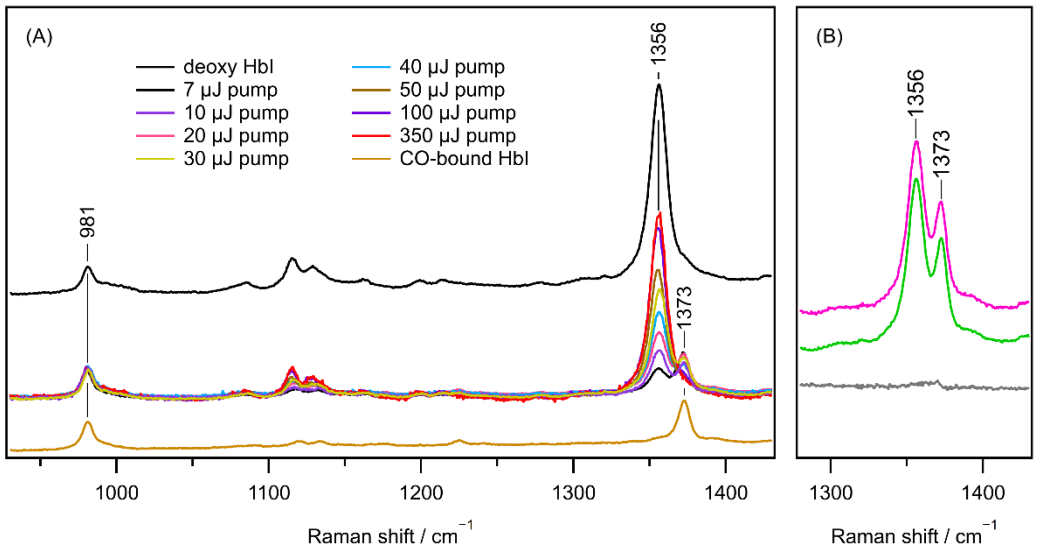

Figure S3. Procedures for determining the dissociation fraction. (A) Raw time-resolved RR spectra of photodissociated HbI at 10 μs measured at various pump pulse energies. The RR spectra of the CO-bound and deoxy forms of HbI are shown for comparison. The pump and probe pulse wavelengths were 532 and 442 nm, respectively. (B) Fit of the raw time-resolved RR spectrum obtained using a 20 μJ pump (magenta) by linear combination of the RR spectra of the CO-bound and deoxy forms [eq. (1), green]. The green trace is vertically shifted for ease of viewing. The gray trace shows residuals of the fitting.

[1]All the spectra were normalized by the band intensity of internal standard Na_2SO_4 at 981 cm^{-1}.

For the determination of the fractions, the two conditions have to be satisfied; (1) the structural transition is fully completed at 10 μs and no other transition would occur, (2) bimolecular rebinding of CO did not yet occur at 10 μs. For the condition (1), we showed that spectral changes can be described by the one-step changes (see the main text). For the condition (2), we described that the condition is satisfied as follows. Figure S4 shows raw time-resolved RR spectra of photodissociated HbI obtained using 30 μJ pump pulses. The ν_4 band of CO-bound HbI at 1373 cm^{-1} showed no recognizable intensity change in the time window. This result demonstrated that the amount of the CO rebinding was negligible in 50 ns–10 μs. Therefore, we determined the dissociation fraction from the ν_4 intensity of the CO-dissociated form at 10 μs delay. Since the band intensity at 1373 cm^{-1} involved contributions not only of the unphotolyzed species but also the CO-rebound species within the instrument response time, we refer to the dissociation fraction determined by this method as the *apparent* dissociation fraction.

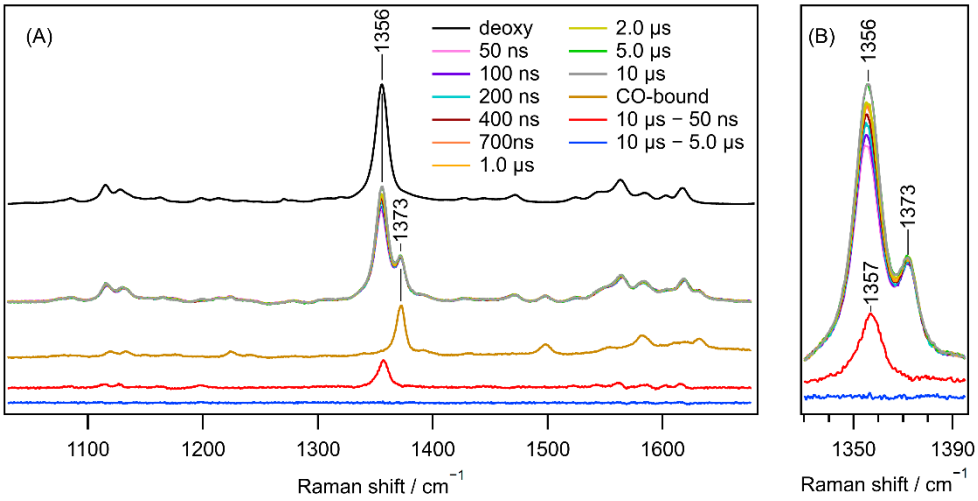

Figure S4. Comparison of the time-resolved RR spectra of photodissociated HbI obtained using 30 μJ pump pulses. (A) Raw time-resolved RR spectra of photodissociated HbI obtained using 30 μJ pump pulses. The RR spectra of the CO-bound (yellow trace) and deoxy forms (black trace) of HbI are shown for comparison. The red trace shows the difference between the spectrum of the delay times at 10 μs and that at 50 ns. The blue trace shows the difference between the spectrum of the delay times at 10 μs and that at 5.0 μs. (B) Expanded views of the ν_4 bands in the raw time-resolved and difference spectra.

S-3. Contributions of the Photodissociated Heme Involved in Singly and Doubly Dissociated HbI to the Time-Resolved RR Spectra

After the photodissociation of HbI at the fraction of f_{dis}, the populational fractions of undissociated HbI, singly dissociated HbI, and doubly dissociated HbI are:

$$P_0 = (1 - f_{\mathrm{dis}})^2 \tag{2}$$

$$P_1 = 2f_{\mathrm{dis}}(1 - f_{\mathrm{dis}}) \tag{3}$$

$$P_2 = {f_{\mathrm{dis}}}^2. \tag{4}$$

The fractions of the spectral contributions by the photodissociated heme involved in singly and doubly dissociated HbI to the time-resolved RR spectra are denoted as f_1 and f_2, respectively. The fractions are given as a function of f_{dis}.

$$f_1 = \frac{P_1}{P_1 + 2P_2} = \frac{2f_{\mathrm{dis}}(1 - f_{\mathrm{dis}})}{2f_{\mathrm{dis}}(1 - f_{\mathrm{dis}}) + 2{f_{\mathrm{dis}}}^2} = 1 - f_{\mathrm{dis}} \tag{5}$$

$$f_2 = \frac{2P_2}{P_1 + 2P_2} = \frac{2{f_{\mathrm{dis}}}^2}{2f_{\mathrm{dis}}(1 - f_{\mathrm{dis}}) + 2{f_{\mathrm{dis}}}^2} = f_{\mathrm{dis}} \tag{6}$$

Note that doubly dissociated HbI contains two dissociated hemes and that its contribution to the time-resolved RR spectra is thus proportional to P_2 times 2.

S-4. Temporal Changes of the Band Frequencies and Intensities of HbI Mutants

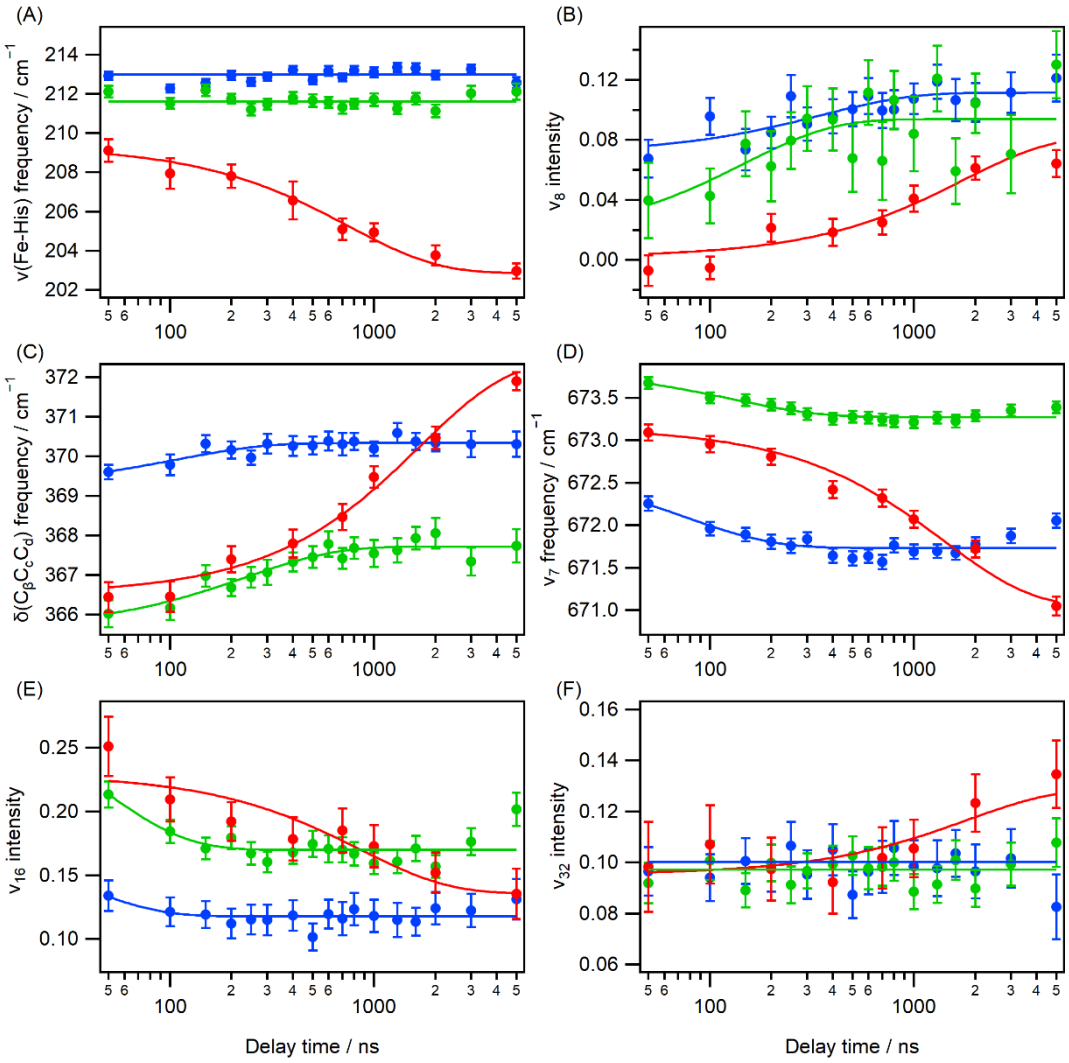

Figure S5. Temporal changes of the band frequencies and intensities of the HbI mutants. (A) ν(Fe–His) frequency, (B) ν_8 intensity, (C) δ($C_\beta C_c C_d$) frequency, (D) ν_7 frequency, (E) ν_{16} intensity, and (F) ν_{32} intensity. Green, blue, and red markers show the frequencies and intensities of the F97Y and F97L mutants, and WT, respectively. Solid lines are the best fits using a single exponential function. The intensities of the ν_8, ν_{16}, and ν_{32} bands are shown as relative values to the intensity of the ν_7 band.

S-5. Comparison Between Protein Structures of HbI and Human Adult Hemoglobin

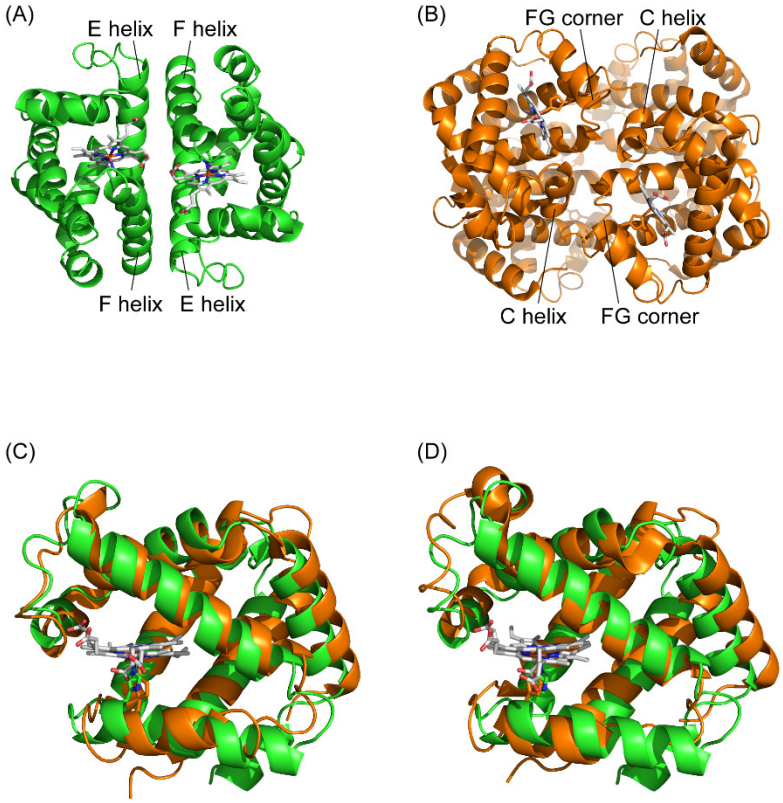

Figure S6. Comparison between protein structures of HbI and human adult hemoglobin (HbA). (A) Molecular structure of deoxy HbI (PDB ID: 4SDH), (B) molecular structure of deoxy HbA (PDB ID: 2HHB). (C) superimposed structures of an HbI subunit and an α chain of HbA, (D) superimposed structures of an HbI subunit and a β chain of HbA. The structures of HbI and HbA are shown in green and orange, respectively. Amino acid similarities of HbI–HbA α chain and HbI–HbA β chain are 25% and 27%, respectively.