

Supporting Information for “Cluster-Dependent Charge-Transfer Dynamics in Iron-Sulfur Proteins”

Ziliang Mao,[†] Shu-Hao Liou,^{†‡} Nimesh Khadka,^{⊥§} Francis E. Jenney Jr.,[⊥] David B. Goodin,[†] Lance C. Seefeldt,[⊥] Michael W. W. Adams,[#] Stephen P. Cramer,^{†} Delmar S. Larsen^{*†}*

[†]Department of Chemistry, University of California Davis, One Shields Avenue, Davis, California 95616, United States

[⊥]Georgia Campus, Philadelphia College of Osteopathic Medicine, Suwanee, Georgia 30024, United States

[#]Department of Biochemistry, The University of Georgia, Athens, Georgia 30602, United States

[⊥]Department of Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, Utah 84322, United States

***Corresponding Authors**

dlarsen@ucdavis.edu & spjcramer@ucdavis.edu

Present Address

[‡]Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, Göttingen 37077, Germany

[§]Department of Pharmacology, Case Western Reserve University, Cleveland, Ohio 44106, United States

Ultrafast Transient Absorption Spectroscopy

The ultrafast transient absorption (TA) technique probes the time-dependent change of sample absorbance upon laser excitation, and reveals information about excited state population evolution, electron and proton transfer, intersystem crossings, and subsequent dynamics. It provides insights into electronic structure, electron transfer properties, and subsequent dynamics on the ultrafast time scale (100 fs - 10 ns).

Global Analysis

Depending on the postulated connectivity between the populations, there are two typical global analysis models.¹ If the populations are assumed to evolve from an initial population consecutively to the subsequent ones ($A \rightarrow B \rightarrow C \rightarrow \dots$), then this model is termed a sequential model and the corresponding population spectra are called evolution-associated difference spectra (EADS). If multiple populations are assumed to be excited to excited states simultaneously and then each of them relax to their respective ground states ($A^* \rightarrow A, B^* \rightarrow B, \dots$), then this model is called a parallel model with the corresponding spectra termed decay-associated difference spectra (DADS). When the postulated model accurately describes the underlying photodynamics, the extracted difference spectra are also called species-associated difference spectra (SADS) and represent the true different spectra of the constituent species/populations in the system of interest. Otherwise, the extracted spectra do not represent the true spectra of the underlying species but a linear combination of the true SADS.

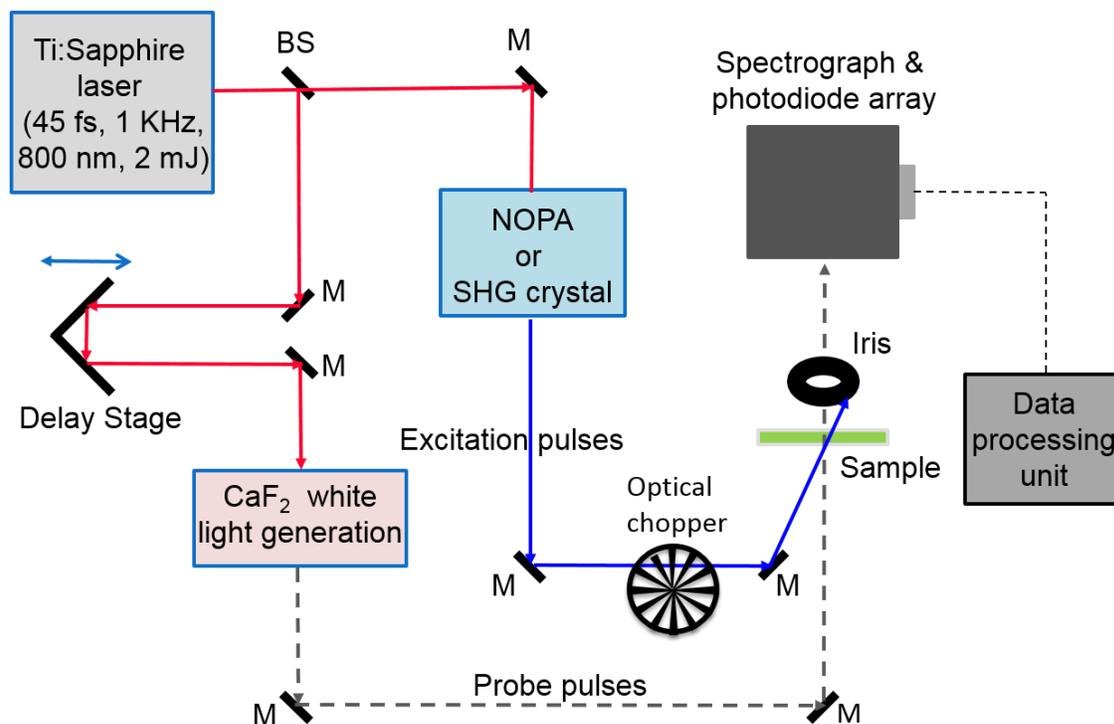


Figure S1. Schematic of the ultrafast transient absorption spectroscopic system. Probe pulses: white light continuum; Excitation pulses: 400 nm for *PfRd*, nitrogenase Fe protein and nitrogenase MoFe protein, 490 nm for Pdx. M: Mirrors or 800 nm /400 nm high reflectors; BS: Beam Splitter; NOPA: Nonlinear Optical Parametric Amplifier; SHG: Second Harmonic Generation; CaF₂: Calcium Fluoride.

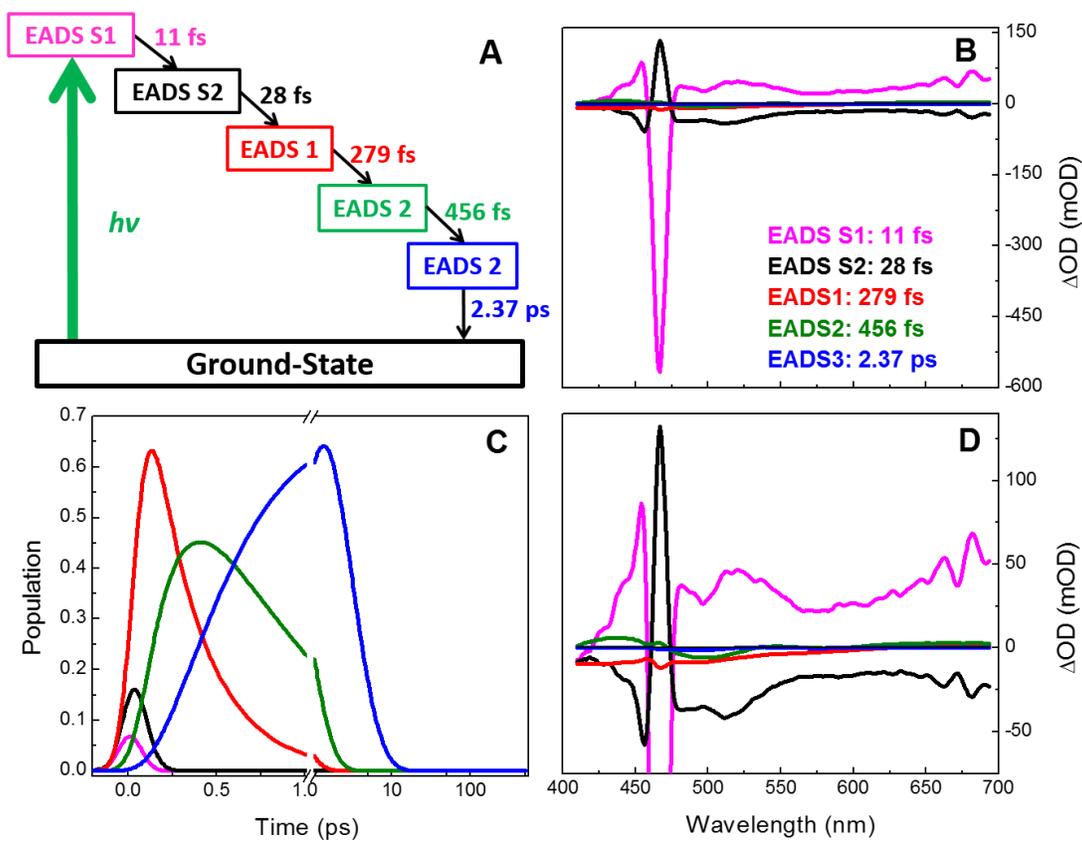


Figure S2. (A) Five-compartment sequential model used to fit the TA data of *PfRd* shown in Figure 4. EADS S1 and EADS S2 are the short-lived components used to fit the cross-phase modulation (CPM) artifacts and water Raman signals in the beginning of the kinetics. (B) Extracted EADS for the fit of the sequential model in (A) to the TA data of *PfRd*. (C) Population evolution profile for the EADS. (D) Zoom-in view of the EADS in (B).

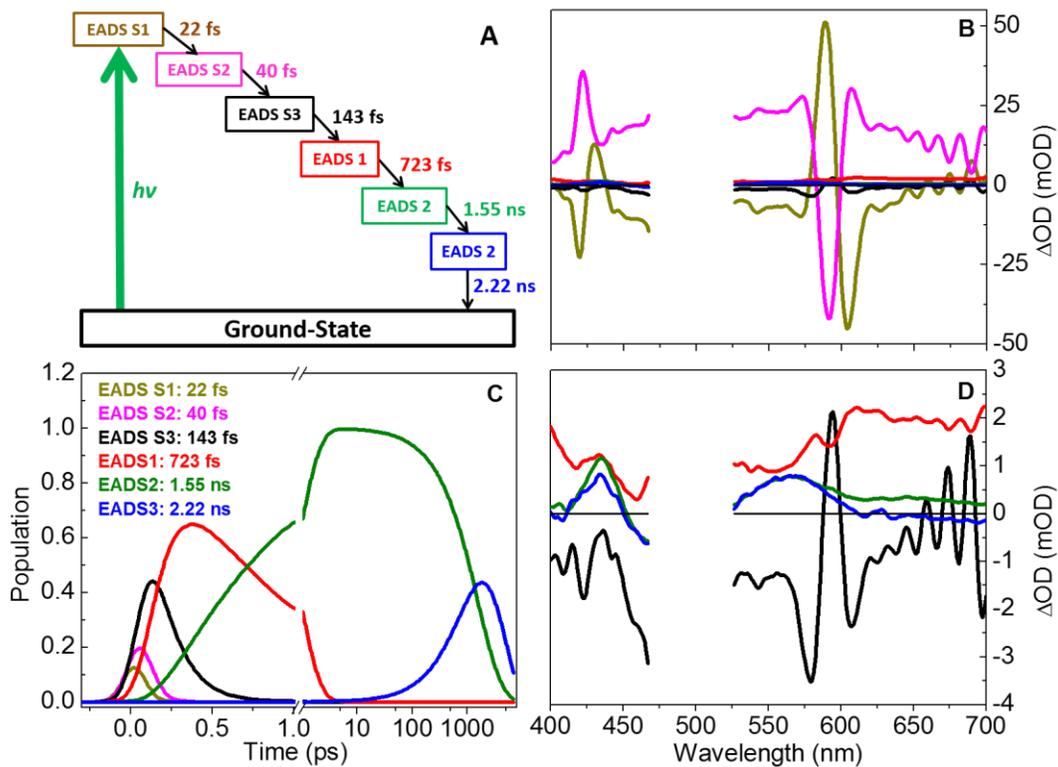


Figure S3. (A) Sequential model used to fit the TA data of Pdx shown in Figure 5. (B) Extracted EADS for the fit of the sequential model in (A) to the TA data of Pdx. EADS S1-3 are the short-lived components used to fit the CPM artifacts and water Raman signals in the beginning of the kinetics. (C) Population evolution profiles for the EADS. (D) Zoom-in view of EADS 1-3 and S3 in (B). The population profiles after 1 ps are on log time scale.

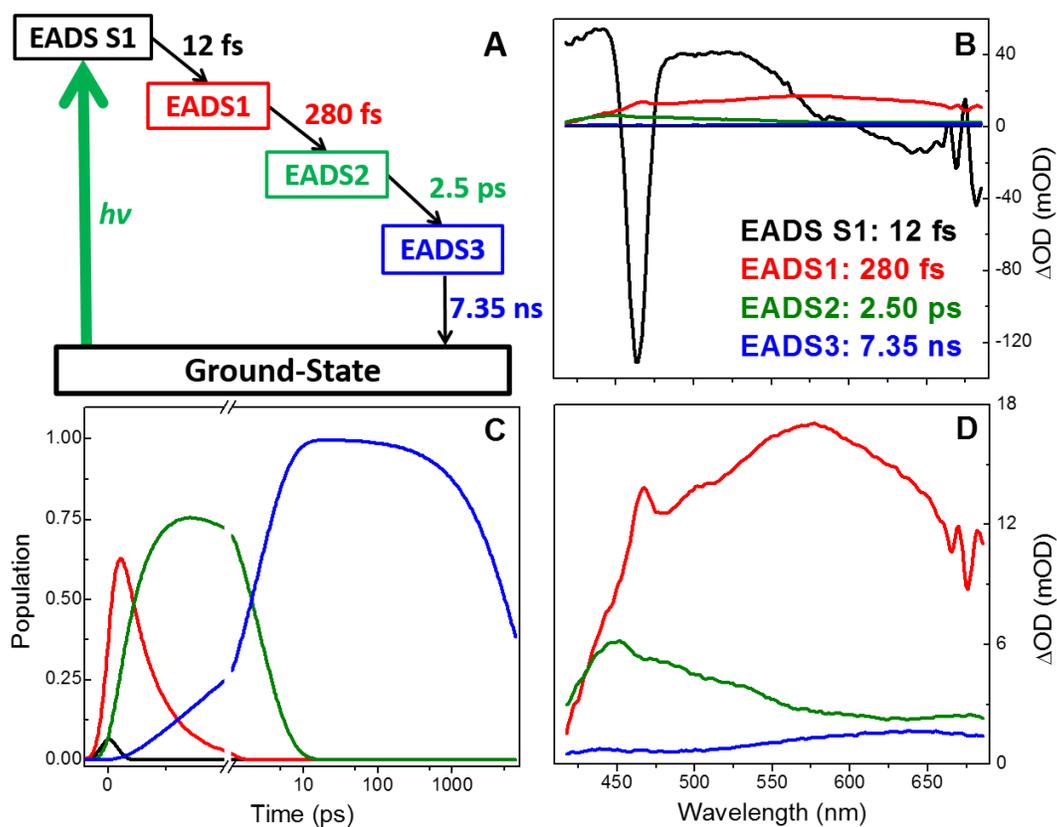


Figure S4. (A) Four-compartment sequential model used to fit the TA data of nitrogenase Fe protein shown in Figure 6. (B) Extracted EADS for the fit of the sequential model in (A) to the TA data of nitrogenase Fe protein. EADS S1 is the short-lived component used to fit the CPM artifacts and water Raman signals in the beginning of the kinetics. (C) Population evolution profiles for the EADS. (D) Zoom-in view of EADS 1-3 in (B). The population profiles after 1 ps are on log time scale.

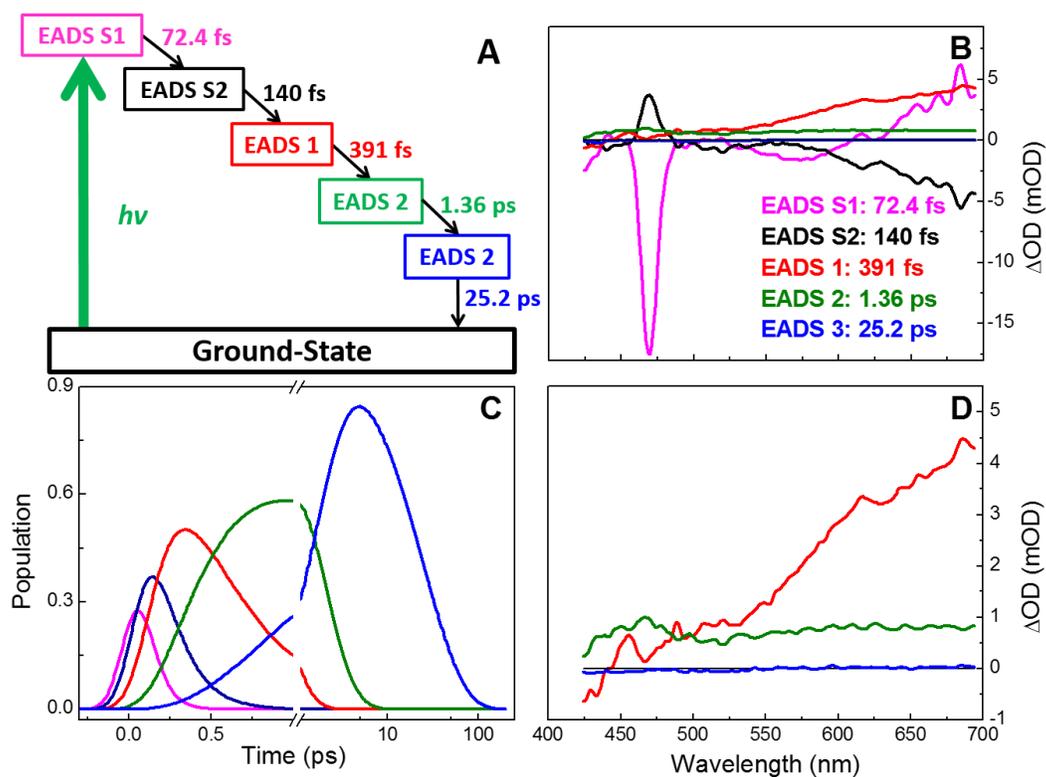


Figure S5. (A) Five-compartment sequential model used to fit the TA data of nitrogenase MoFe protein shown in Figure 7. (B) Extracted EADS for the fit of the sequential model in (A) to the TA data of nitrogenase MoFe protein. EADS S1 and S2 are used to fit the shorted-lived cross-phase modulation artifact and water Raman signals. (C) Population evolution profiles for the EADS. (D) Zoom-in view of EADS 1-3 in (B). The population profiles after 1 ps are on log time scale.

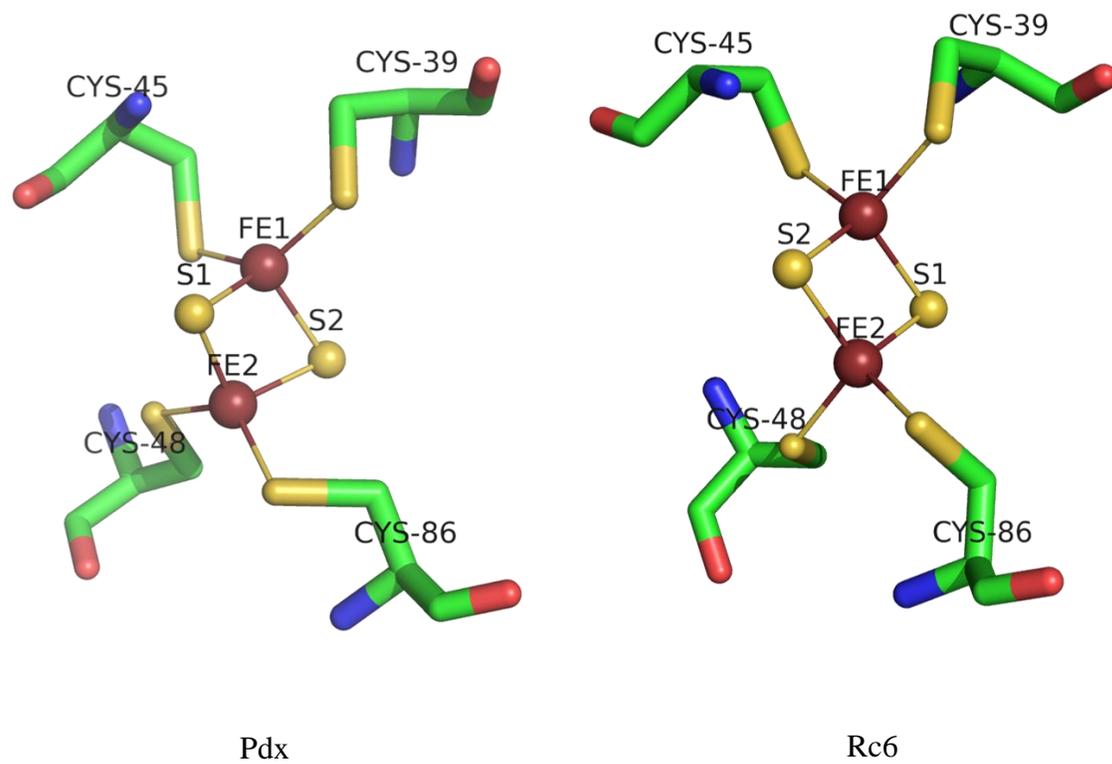


Figure S6. Active site structures of Pdx (Left) and Rc6 (Right). Color coding: Fe (brick), S (yellow), C (green), N (blue), O (red). PDB codes: 1PUT (Pdx), 1E9M (Rc6).

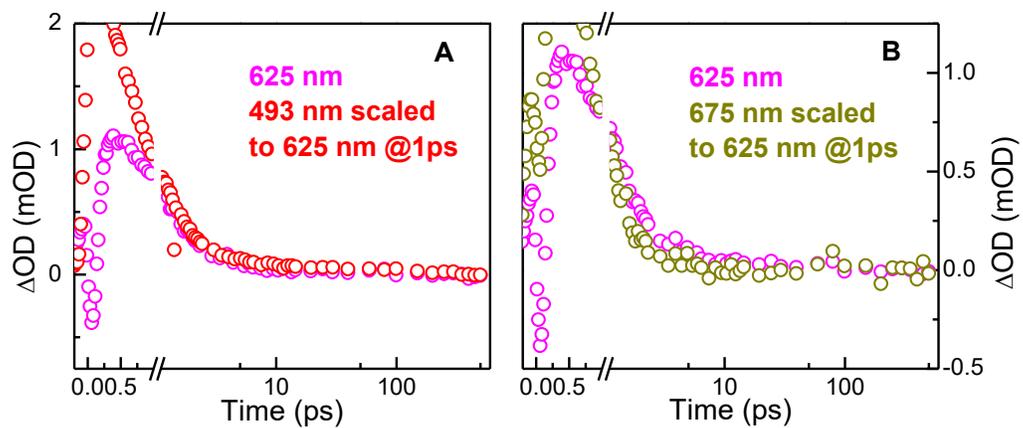


Figure S7. Comparison of the *PfRd* kinetics at 493 nm, 625 nm and 675 nm after 1 ps. The data after 1 ps is on log time scale.

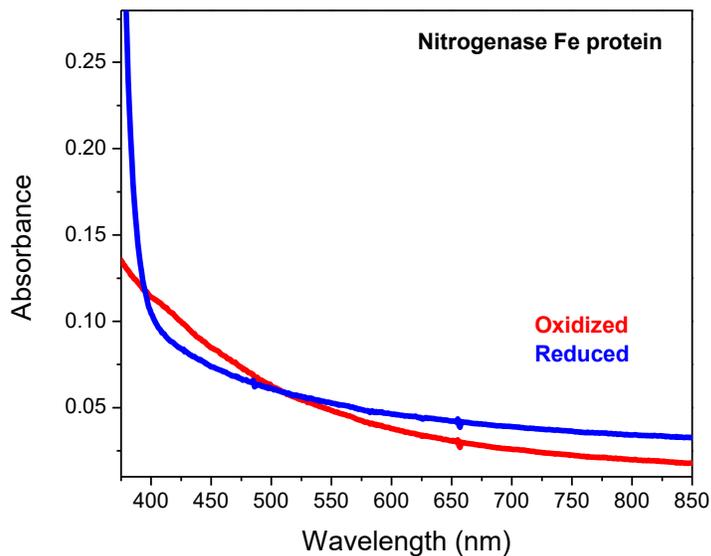


Figure S8. Steady-state UV-Vis absorption spectra of oxidized (red) vs sodium dithionate reduced (blue) nitrogenase Fe protein. The region lower than 375 nm was cut off because of the strong dithionate signal present in that region in the reduced protein spectrum. Optical pathlength: 1 cm; Fe protein concentration: 10 μ M; sodium dithionate concentration: 5 mM.

References

- [1] van Stokkum, I. H. M., Larsen, D. S., and van Grondelle, R. (2004) Global and target analysis of time-resolved spectra, *Biochim. Biophys. Acta, Bioenerg.* 1657, 82-104.