

Direct Measurement of the Tryptophan-Mediated Photocleavage Kinetics of a Protein Disulfide Bond

Supporting Information

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CD Measurements of Wild-Type Z34C and Trp-Z34C

For the CD results presented in Figure S1, the optical densities of both samples at 266 nm were kept equivalent. To do so, the concentration (83 μM) of the wild-type Z34C sample was higher than that (13 μM) of the Trp-Z34C sample. Both samples were irradiated for 2 hrs. As can be seen, upon irradiation, the molar ellipticity of the wild-type Z34C sample decreased by $\sim 2 \times 10^3$ deg cm^2 dmol^{-1} , whereas that of the Trp-Z34C sample decreased by $\sim 5 \times 10^3$ deg cm^2 dmol^{-1} at 222 nm.

Estimating the Triplet State Quenching Rate by Disulfide

Using the software of VMD, the distance between the tryptophan (Trp) residue and the disulfide bond in Trp-Z34C was estimated to be between 5 – 10 Å. Using this distance as the diameter of a sphere wherein a single pair of Trp and disulfide resides, the effective quencher concentration was estimated to be 3.17 – 25 M. Lapidus *et al.*¹ have shown that the bimolecular quenching rate constant of the triplet state of Trp by a disulfide is 1.9×10^8 M^{-1} s^{-1} . Using this value and the determined effective quencher concentration, we estimated the triplet state quenching rate by the disulfide in Trp-Z34C to be (0.2 – 2 ns)⁻¹.

Removal of Solvent Contribution to the Kinetic Offset

The absorption spectrum of D₂O in the amide I' region depends on temperature. As discussed in the main text, photoexcitation of Trp with a 266 nm ns pulse leads to an increase in the sample temperature and hence a long-lived D₂O signal due to this dependence. To subtract the D₂O signal, we collected the absorption spectra of D₂O in the amide I' region at different temperatures and, based on these data, we can calculate the D₂O signal for a given set of known parameters, i.e., frequency, pathlength, and temperature change (i.e., ΔT). In the current case, ΔT was estimated to be ~ 0.3 °C, using the transient kinetic curve obtained at 1652 cm^{-1} , which is dominated by the temperature jump signal. Using this information and also the optical pathlength of the sample cell used in UV-pump and IR-probe measurements, we can determine the D₂O contribution to the kinetic offset at each probing frequency.

Electron Scavenger Experiment

In this experiment, two Trp-Z34C samples of identical concentrations (~ 40 μM), with and without the presence of N₂O, were used. The N₂O was introduced by bubbling pure N₂O gas through the sample for at least 20 minutes, which was shown to accrue a sufficient amount of N₂O as determined by FTIR. These samples were then subject to UV light irradiation in 1 hr intervals with shaking of the sample holder every 30 min. The CD spectra of these samples were then taken at different time points and the results are shown in Figure S3.

Free Thiol Determination

UV-Vis spectra shown in Figure S5 were collected on a V-650 UV-Vis spectrophotometer (Jasco, MD) using a 1 cm quartz cuvette. First, the UV-Vis spectrum of a 1.6 mM solution of an Ellman's reagent, 5,5'-dithio-bis-[2-nitrobenzoic acid], in a 20 mM sodium phosphate buffer (pH 7) was recorded. Second, a Trp-Z34C sample solution that had been irradiated with 266 nm light for ~15 hrs was added to this solution to yield a final protein concentration of 8 μ M. Third, the UV-Vis spectrum of this mixture was taken. As shown (Figure S5), the change in absorbance at 412 nm was \sim 0.06, which indicates that the concentration of 2-nitro-5-thiobenzoate dianion (TNB²⁻), a product of the reaction between the Ellman's reagent and a free thiol, is \sim 4 μ M, determined using the molar extinction coefficient of TNB²⁻ at 412 nm ($\epsilon = 14,150 \text{ M}^{-1} \text{ cm}^{-1}$).

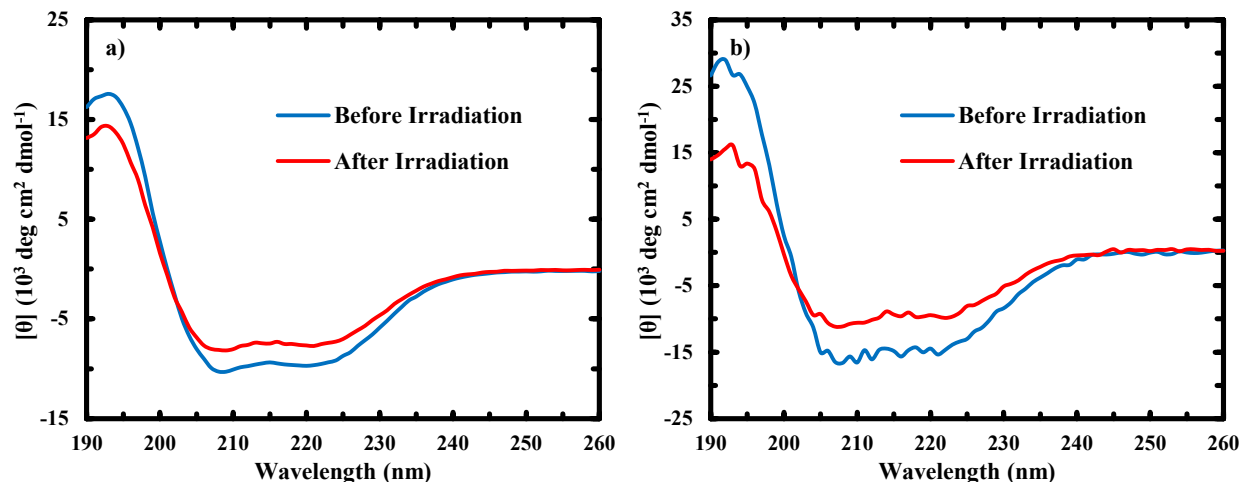


Figure S1. CD spectra of wild-type Z34C (a) and Trp-Z34C (b) taken before and after irradiation with 266 nm light for 2 hrs, as indicated.

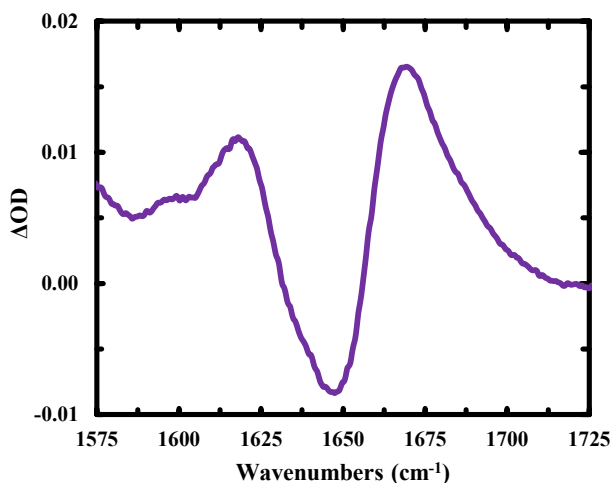


Figure S2. FTIR difference spectrum of Trp-Z34C in the amide I' band region. This spectrum was generated by subtracting the FTIR spectrum of a non-irradiated Trp-Z34C sample (2.8 mM in D₂O buffer at pH 7) from that of the same sample irradiated for 2.5 days.

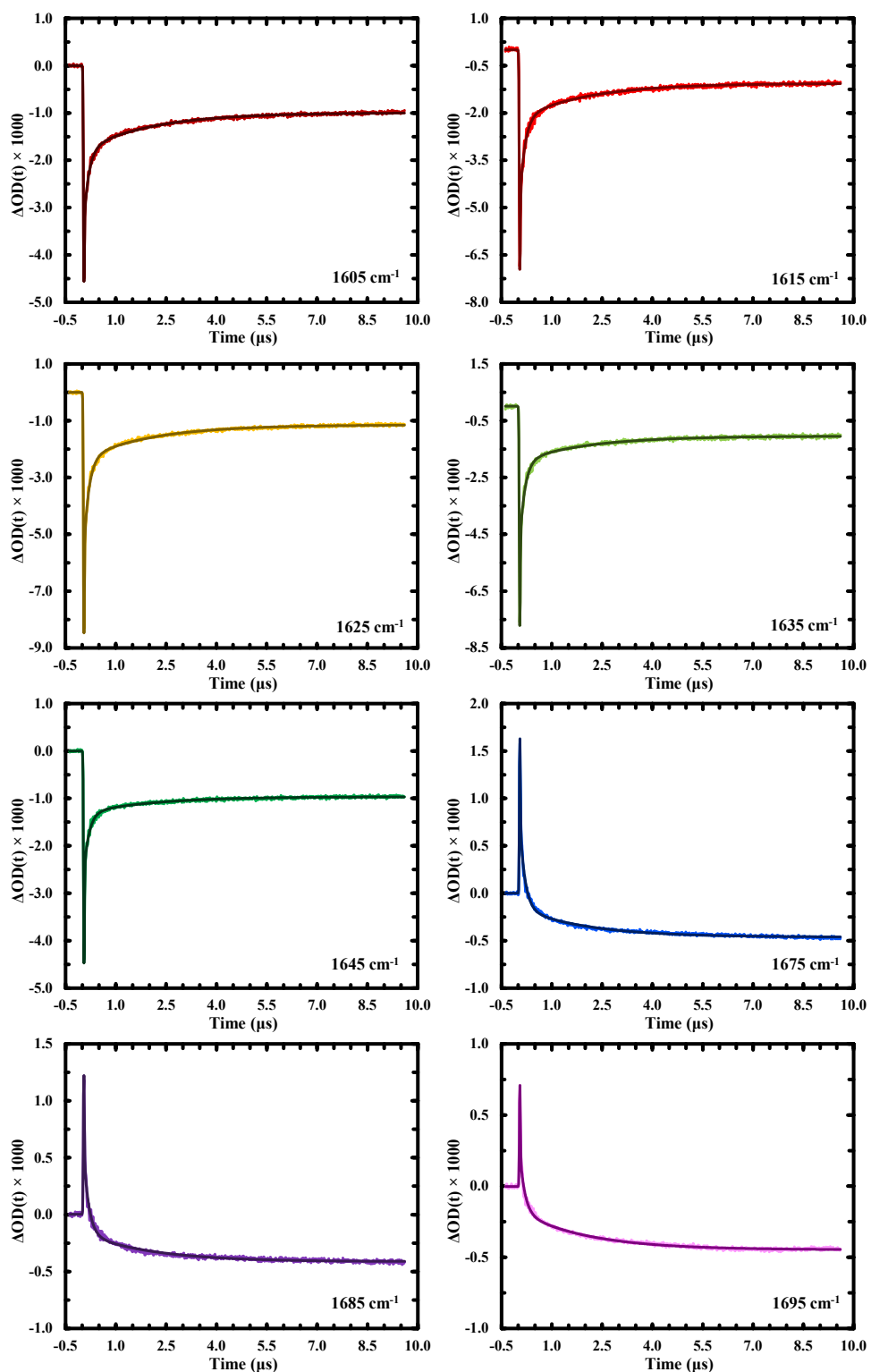


Figure S3. IR transient kinetic traces of Trp-Z34C obtained at the probing frequencies indicated. The smooth lines are global fits of these traces to a three-exponential function plus an offset with the following time constants: 7 ns, 151 ns, and 2.0 μ s. The amplitudes of the four kinetic components are listed in Table S1.

Table S1. Amplitudes (in mOD) of the four kinetics components at different probing frequencies.

Time Constants	Amplitudes of Kinetic Phases									
	1605 cm ⁻¹	1615 cm ⁻¹	1625 cm ⁻¹	1630 cm ⁻¹	1635 cm ⁻¹	1645 cm ⁻¹	1664 cm ⁻¹	1675 cm ⁻¹	1685 cm ⁻¹	1695 cm ⁻¹
Offset	-1.00 ± 0.002	-1.06 ± 0.01	-1.15 ± 0.01	-1.11 ± 0.04	-1.05 ± 0.01	-0.96 ± 0.01	-0.51 ± 0.02	-0.46 ± 0.02	-0.417 ± 0.003	-0.445 ± 0.004
2.0 ± 0.2 μs	-0.79 ± 0.07	-1.1 ± 0.3	-1.2 ± 0.1	-1.0 ± 0.7	-0.9 ± 0.1	-0.35 ± 0.04	0.26 ± 0.06	0.30 ± 0.07	0.25 ± 0.03	0.26 ± 0.02
150 ± 50 ns	-1.6 ± 0.3	-2.8 ± 0.5	-3.3 ± 0.5	-3.0 ± 0.4	-2.9 ± 0.4	-1.3 ± 0.3	0.71 ± 0.07	0.9 ± 0.2	0.7 ± 0.1	0.42 ± 0.06
7 ± 3 ns	-5.7 ± 0.4	-9.2 ± 0.5	-12.0 ± 0.8	-11.9 ± 0.8	-11.4 ± 0.7	-7.1 ± 0.6	2.2 ± 0.2	3.0 ± 0.3	2.37 ± 0.09	1.6 ± 0.2

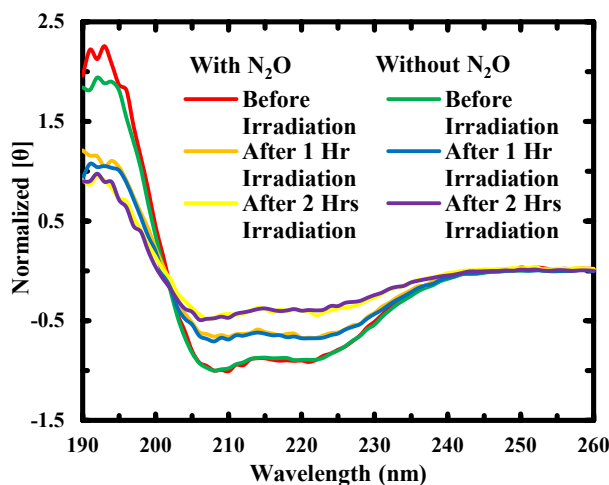


Figure S4. Effect of an electron scavenger (N₂O) on the photoinduced disulfide cleavage in Trp-Z34C, assessed via CD spectroscopy. These normalized CD spectra of two Trp-Z34C samples, one containing N₂O and the other with no electron scavenger, show that the presence of N₂O has no significant effect on the disulfide cleaving efficiency.

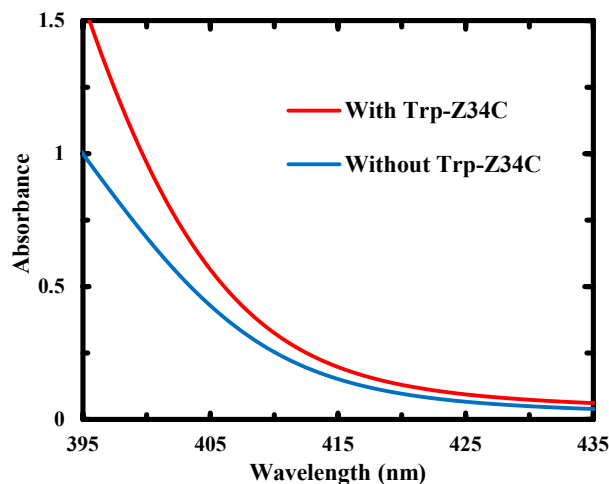


Figure S5. UV-Vis spectra of an Ellman's reagent sample with and without the presence of light-irradiated Trp-Z34C peptide, as indicated.

References

1. L. J. Lapidus, W. A. Eaton and J. Hofrichter, *Proc. Natl. Acad. Sci.*, 2000, **97**, 7220–7225.