Supporting Information

Analysis of the Temperature Dependence of Picosecond-Resolved Fluorescence Properties at Two Functionally Distinct Tryptophans in a Thermophilic Alcohol Dehydrogenase

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Table S1. Bimolecular Quenching Constants Derived from Stern-Volmer Plots.

Temperature, °C	W87in Quenching Constant, $M^{-1}s^{-1}$ *10^{-8}	W167in Quenching Constant, $M^{-1}s^{-1}$ *10^{-8}
10	1.99 (0.05)	3.21 (0.06)
15	2.44 (0.24)	3.93 (0.16)
20	2.52 (0.03)	4.58 (0.16)
25	2.74 (0.20)	5.48 (0.49)
30	3.19 (0.11)	5.72 (0.12)
35	3.46 (0.40)	6.59 (0.25)
40	3.82 (0.23)	7.81 (0.33)
45	4.54 (0.51)	9.47 (0.74)
50	5.15 (0.49)	10.30 (0.12)

Figure S1. Excitation spectra of W87in with increasing NADH titrated in 1 μM increments (from 0–12 μM). Emission was monitored at 410 nm. Arrows indicate the intensity observed with increasing NADH. The peak at ca. 290nm represents the tryptophan absorption, and the peak at 340nm represents NADH cofactor.

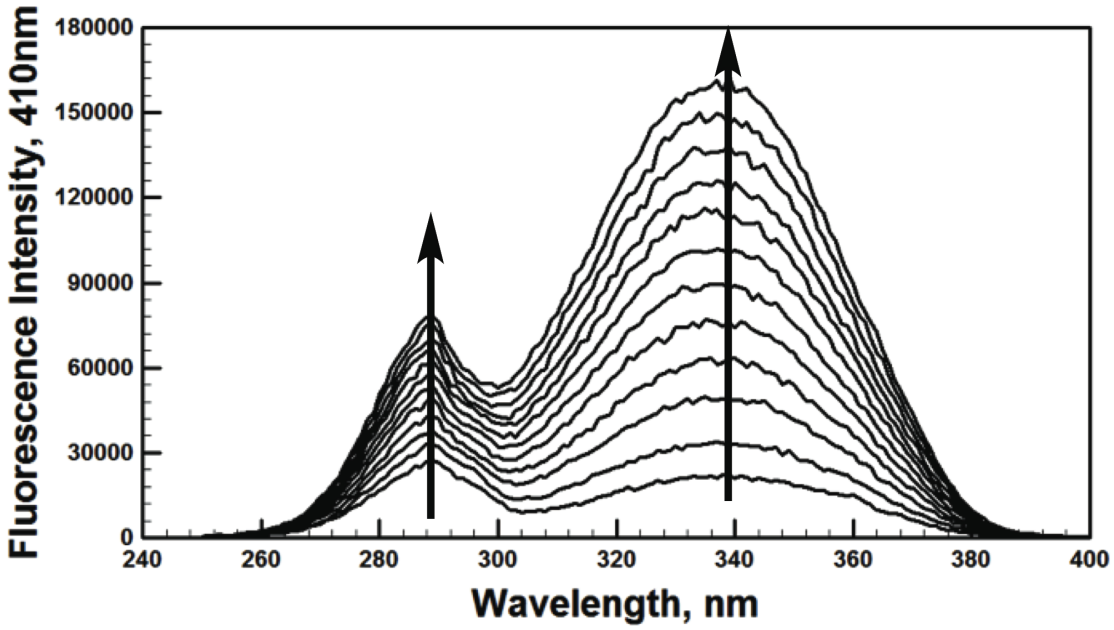
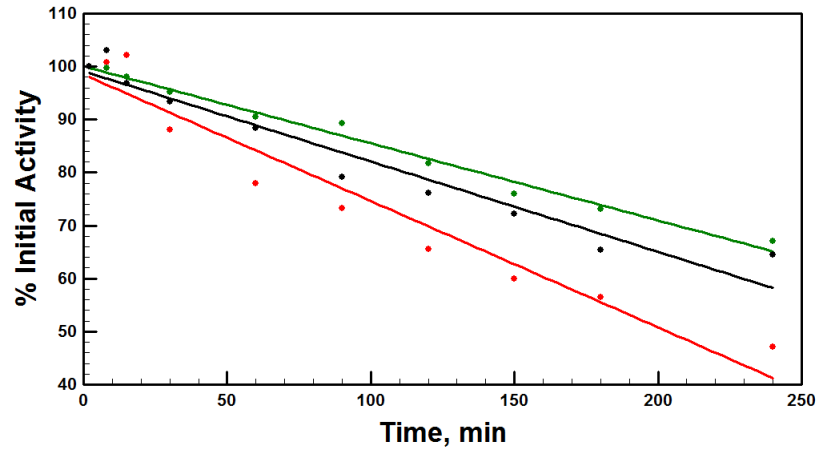


Figure S2. Time-dependent inactivation kinetics at 4 μM W87in (top) and W167in (bottom) at 10ºC (black), 30ºC (green), 50ºC (red). The dashed line illustrates the maximum time the samples were used for data acquisition at a given temperature before replacing with a fresh sample.



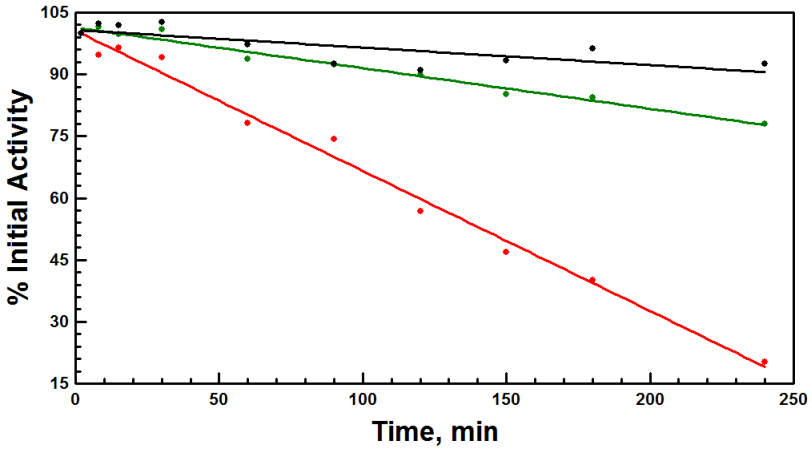


Figure S3. CD spectra of WT (blue), W87in (green), and W167in (magenta) at 30ºC.

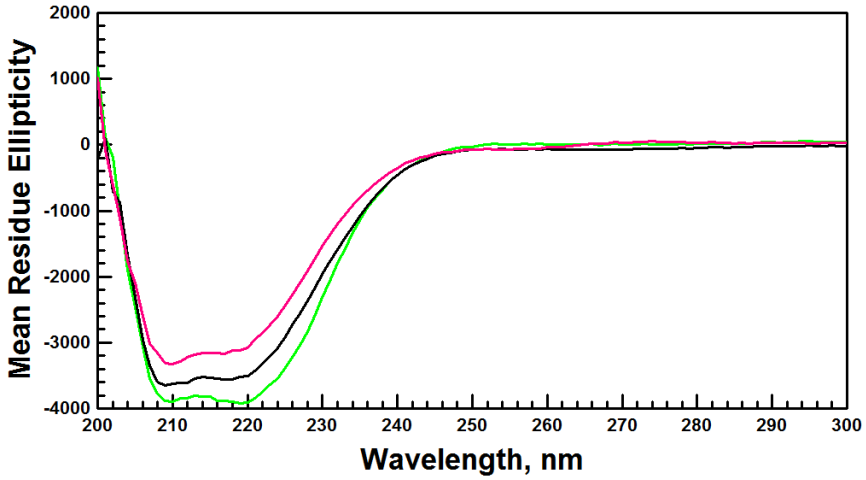


Figure S4. FPLC chromatogram of WT (black), W87in (green), and W167in (magenta). At 4ºC. Protein concentration is at 32 μM.

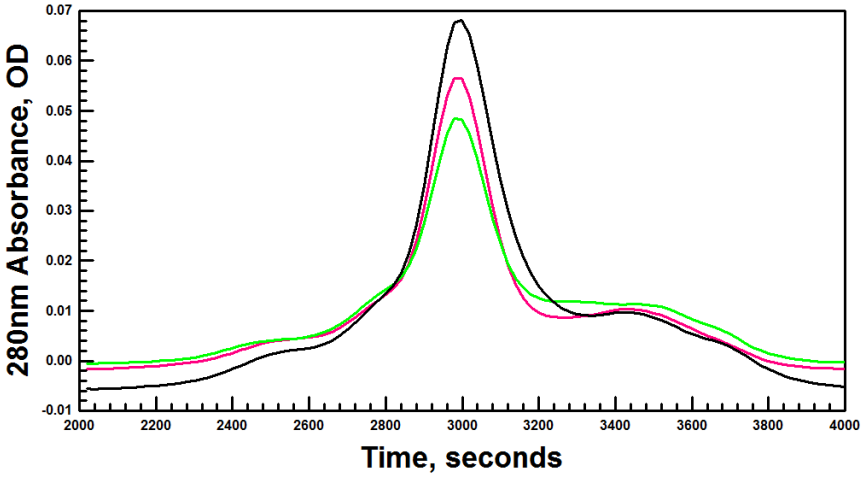


Figure S5. Arrhenius plots for WT (Black), W87in(red), and W167in (blue).

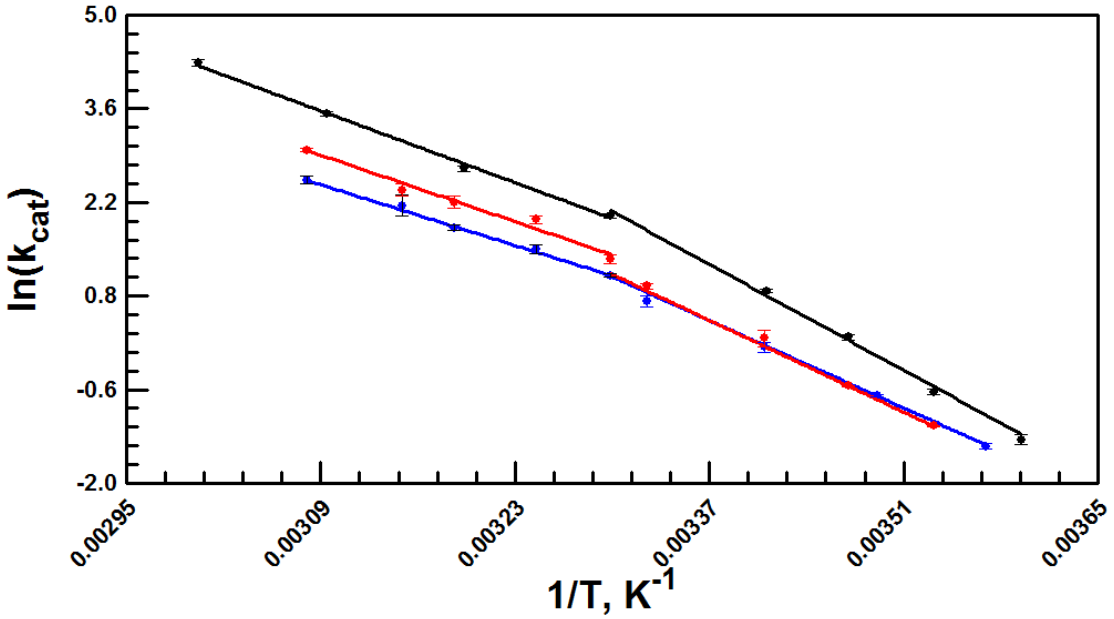


Figure S6. Peak-normalized steady-state emission spectra for W87in (top) and W167in (bottom). All values are normalized to the peak intensity at 10ºC, the temperature of the highest emission intensity. Spectra are shown for 10ºC (black), 20ºC (blue), 30ºC (green), 40ºC (orange), and 50ºC (red).

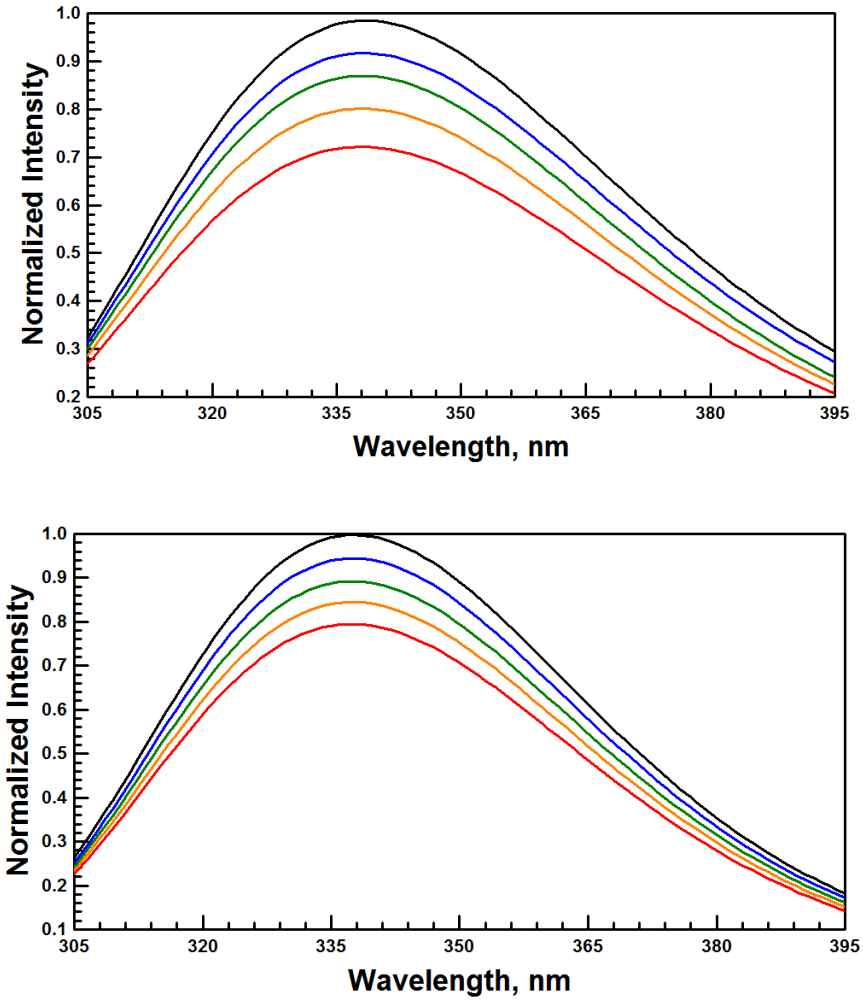


Figure S7. Temperature dependence of the peak emission wavelength in W87in (top) and W167in (bottom).

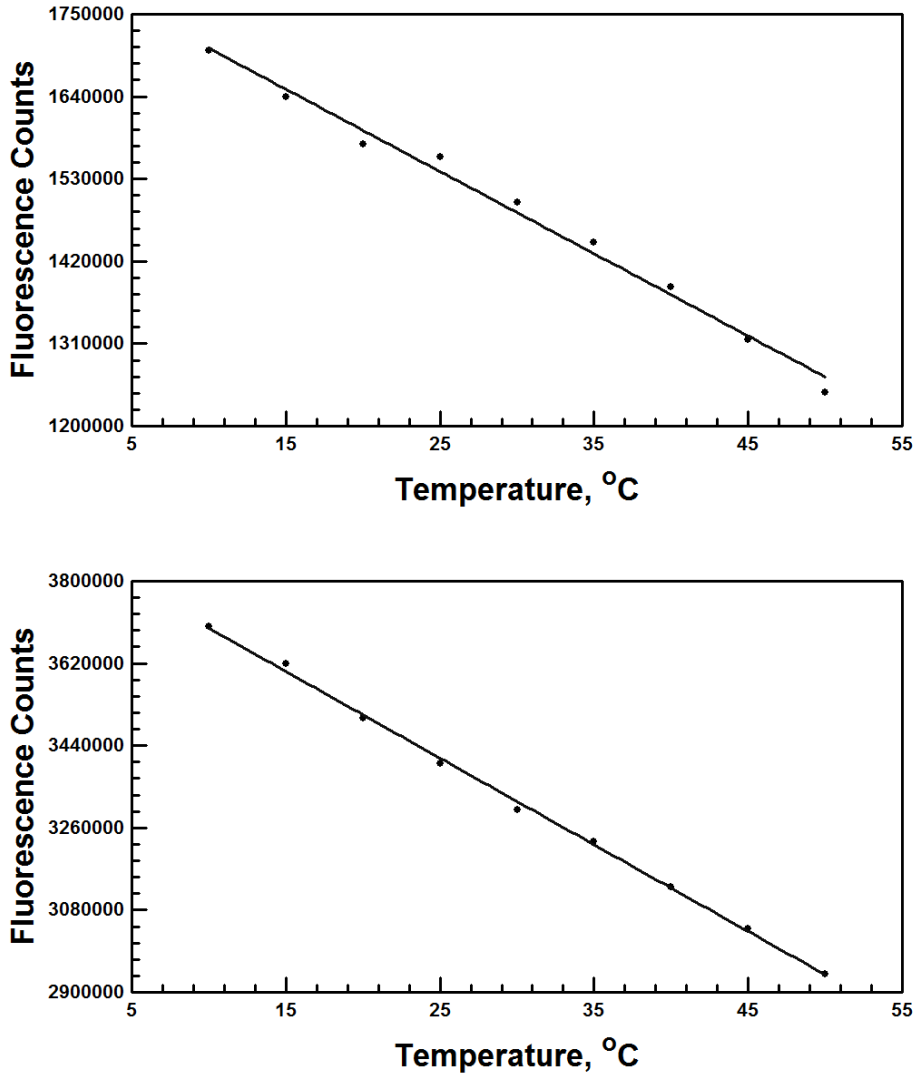


Figure S8. Arrhenius plots for the short (blue) and long (red) lifetime components in W167in.