Supporting Information

Picosecond-Resolved Fluorescence Studies of Substrate and Cofactor-Binding Domain Mutants in a Thermophilic Alcohol Dehydrogenase Uncovers an Extended Network of Communication

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Figure S1. Time-dependent deactivation profiles for the six mutants investigated at 10ºC (black), 30ºC (green), and 50ºC (red). Data for W87in:Y25A and W167in:Y25A are only shown at 10ºC (black) and 45ºC (red) because of the absence of a break. Fresh samples were introduced to the fluorimeter after 25% of the original activity was lost.

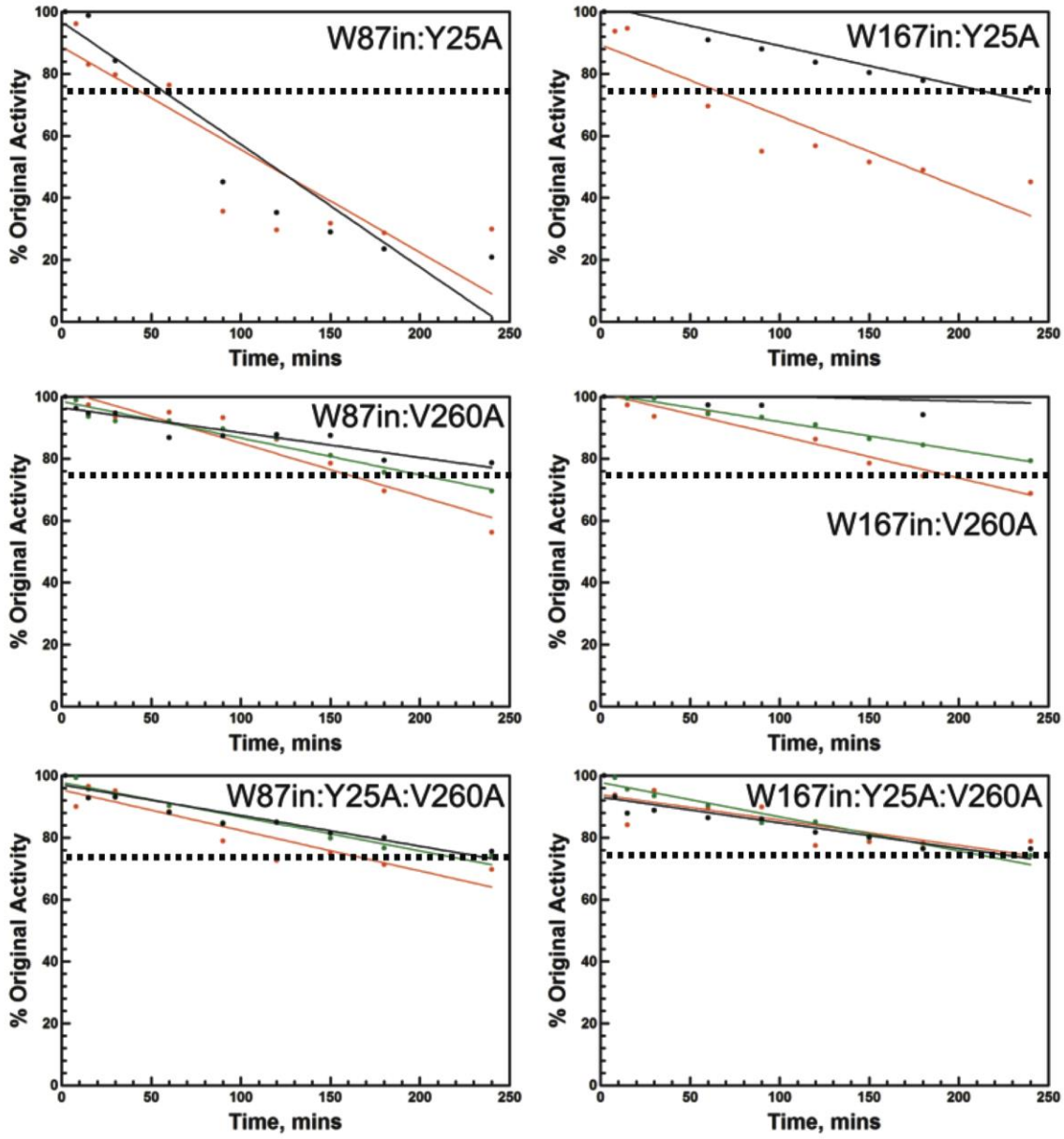

Figure S2. Gel filtration chromatograms of all mutants studied. Each chromatogram contains the W87in (red) and W167in (blue) construct with the additional mutations indicated in each chromatogram's inset. Elution profiles were collected at 4°C.

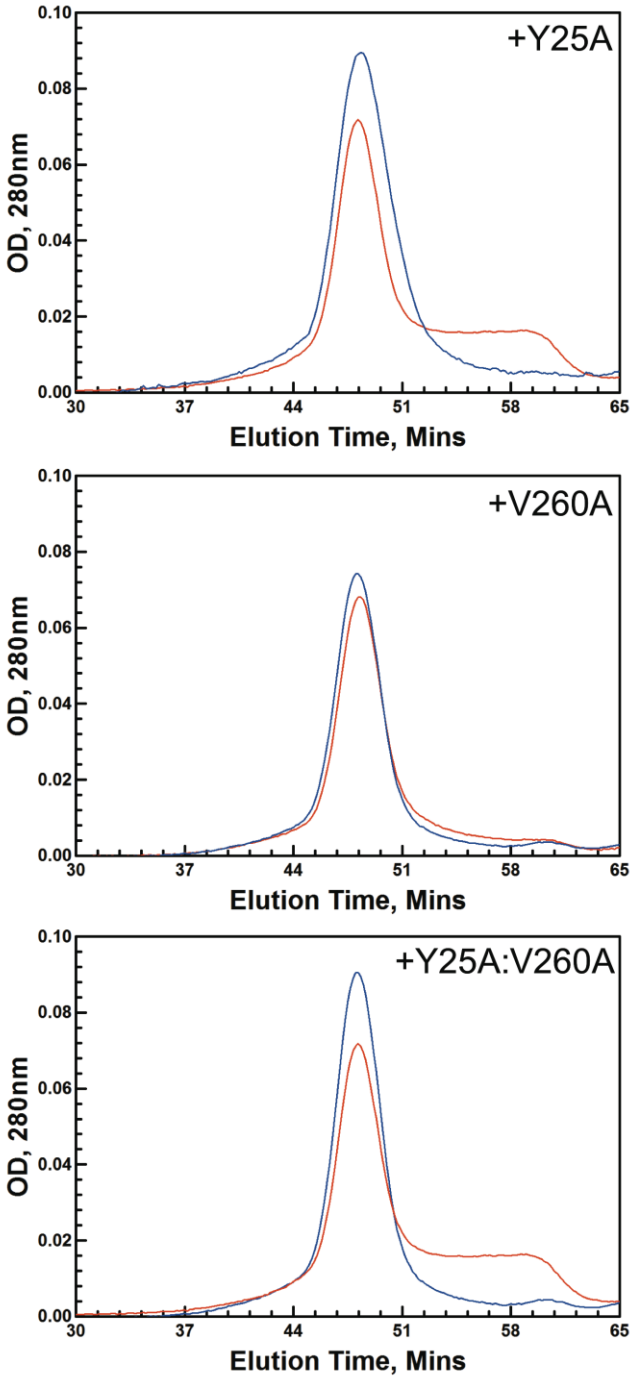

Figure S3. Arrhenius plots measuring the temperature dependence of k_{cat} for the mutants in this study. Each plot contains the W87in (red) and W167in (blue) construct with the additional mutations indicated in the inset of each Arrhenius plot.

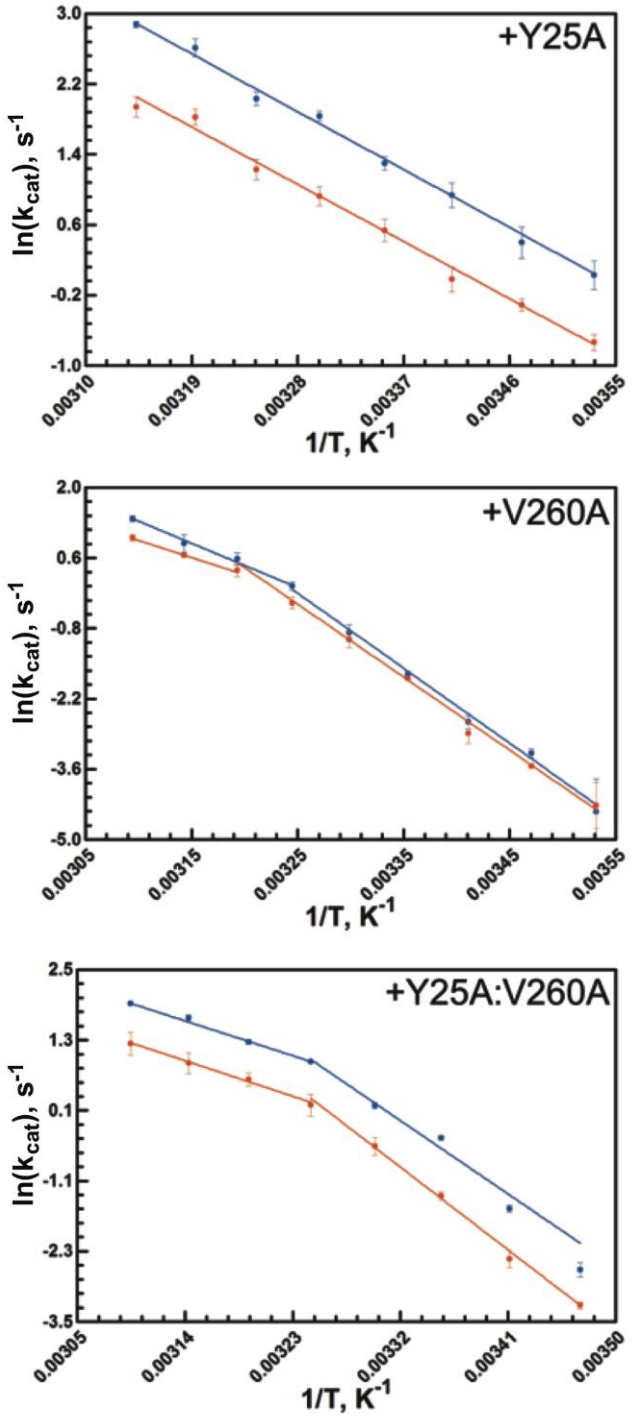

Figure S4. Steady-state emission spectra collected as a function of temperature in the W87in and W167in mutant series. Spectra are shown at 10ºC (black), 20ºC (blue), 30ºC (green), 40ºC (orange), and 50ºC (red).

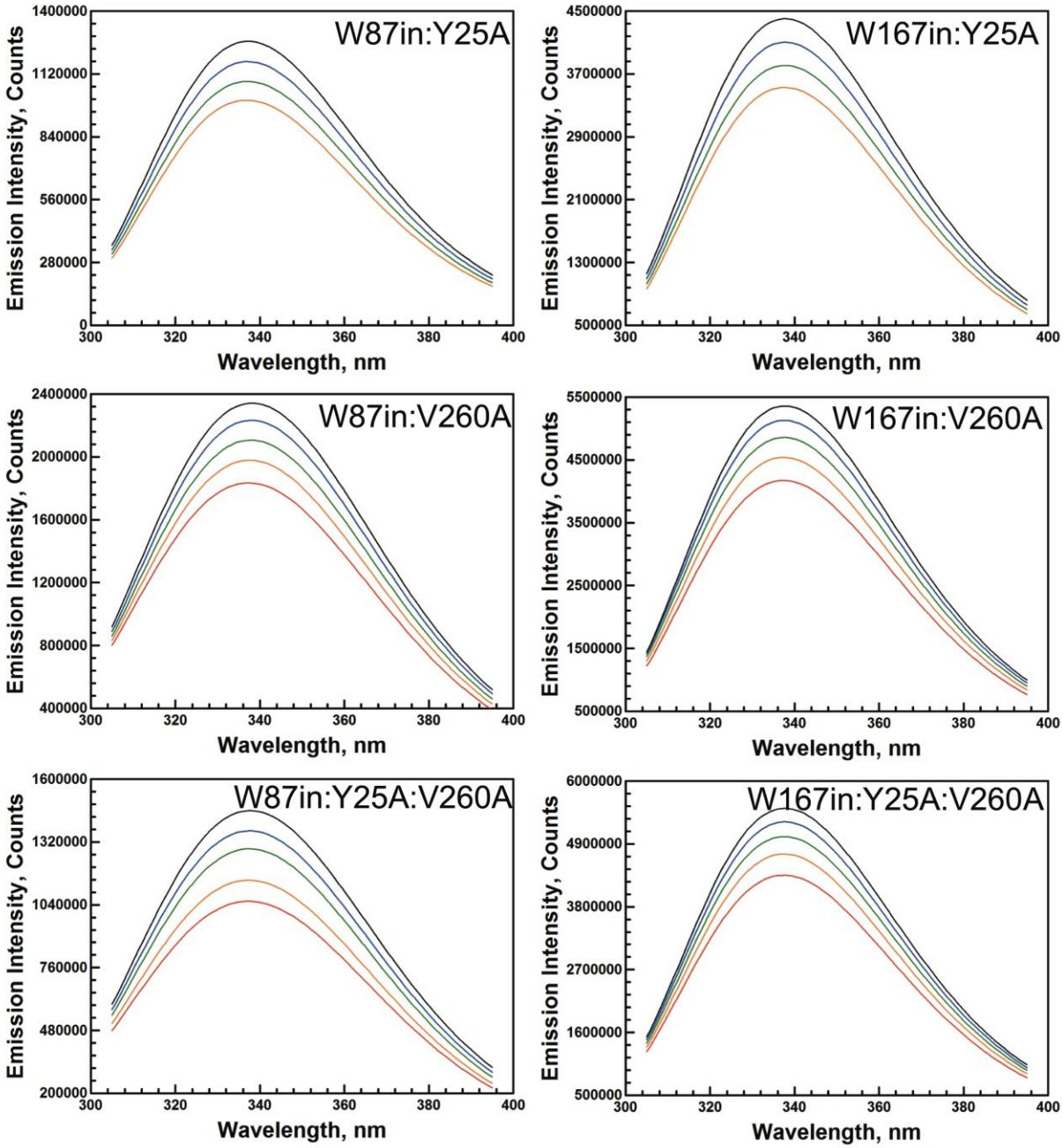

Figure S5. Peak emission intensity plotted as a function of temperature for all six mutants. The constructs are shown as W87in (red) and W167in (blue) with the additional mutations indicated in each graph's inset.

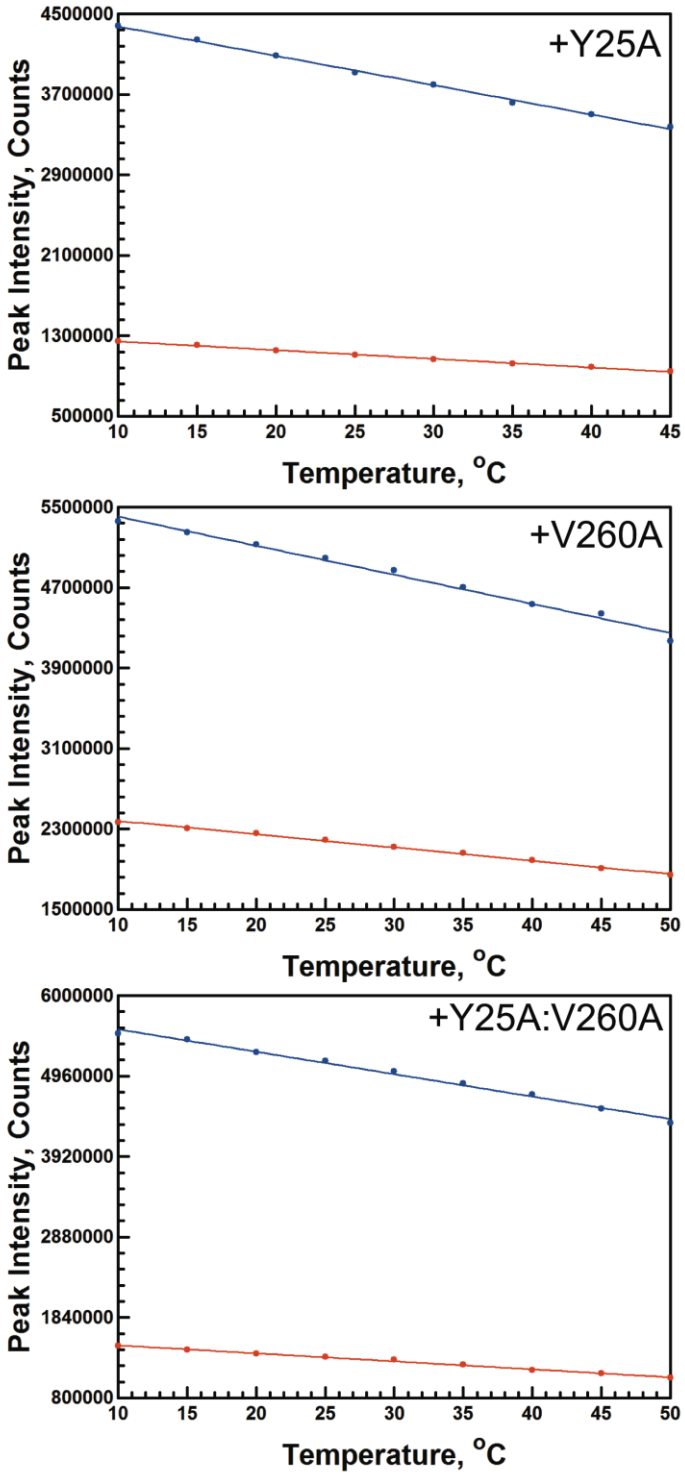

Figure S6. Temperature dependence of the W87in:Y25A fluorescence lifetime decay components. τ_1 is shown in black, τ_2 is shown in red, and τ_3 is shown in blue. It should be noted that the relative scatter for τ_1 component is higher than measured for W87in.

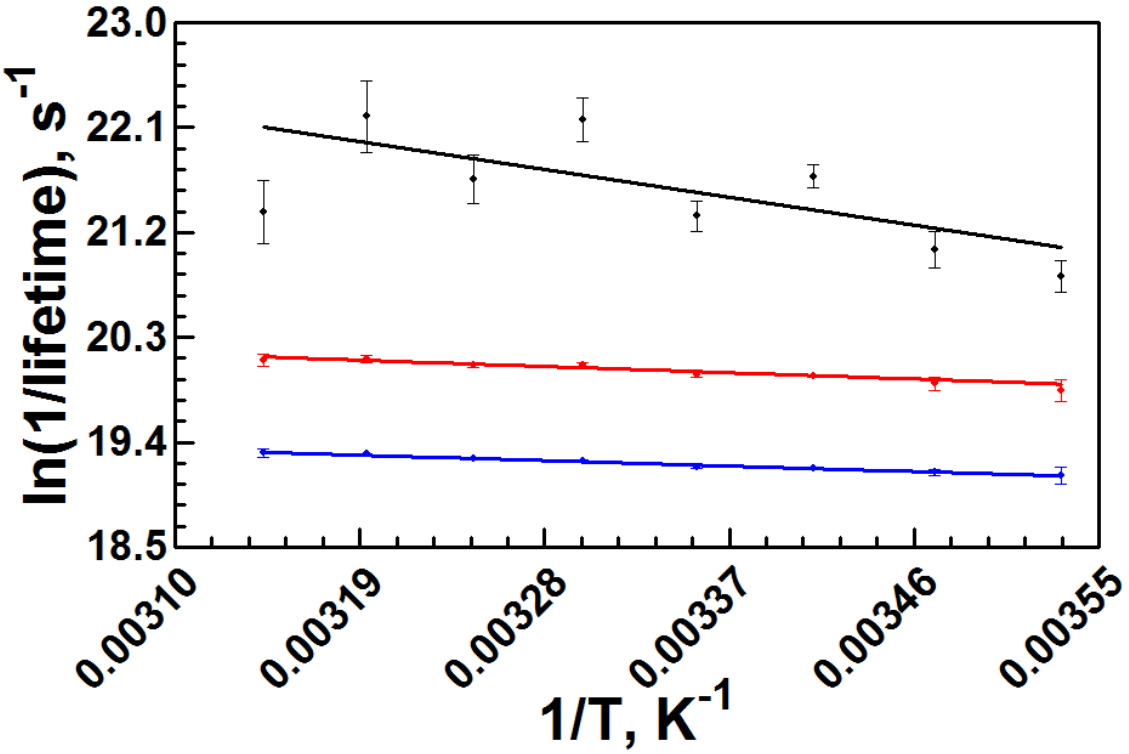

Figure S7. Selected time-resolved emission spectra for the W87in mutant series at 30°C. The spectral slices shown signify the percentage of the red shift completed at 0% (black), 33% (periwinkle), 67% (lime) and ca. 100% (magenta). The times at which each spectrum occurs vary, and are more fully characterized in Figure 4 and Table 4. The additional mutation is noted within each inset.

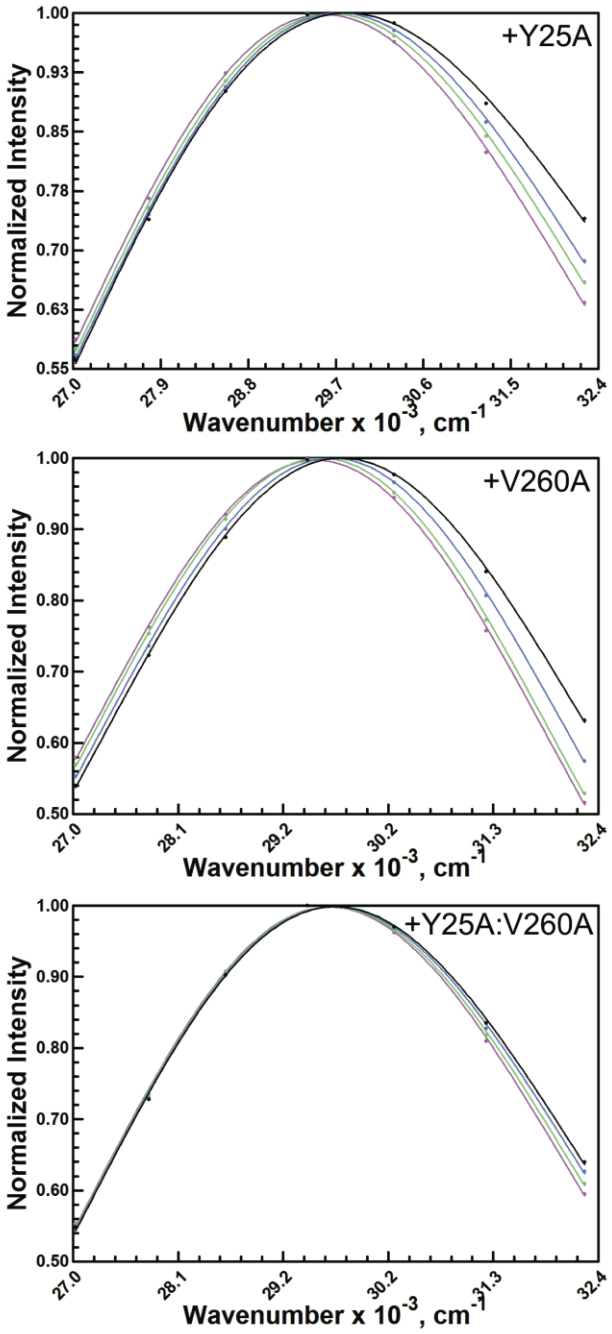

Figure S8. Selected time-resolved emission spectra for the W167in mutant series at 30°C. Each additional mutation is indicated within each inset. The spectral slices shown signify the percentage of the red shift completed at 0% (black), 33% (periwinkle), 67% (lime) and ca. 100% (magenta). The times at which each spectrum occurs vary, and are more fully characterized in Figure 4 and Table 4.

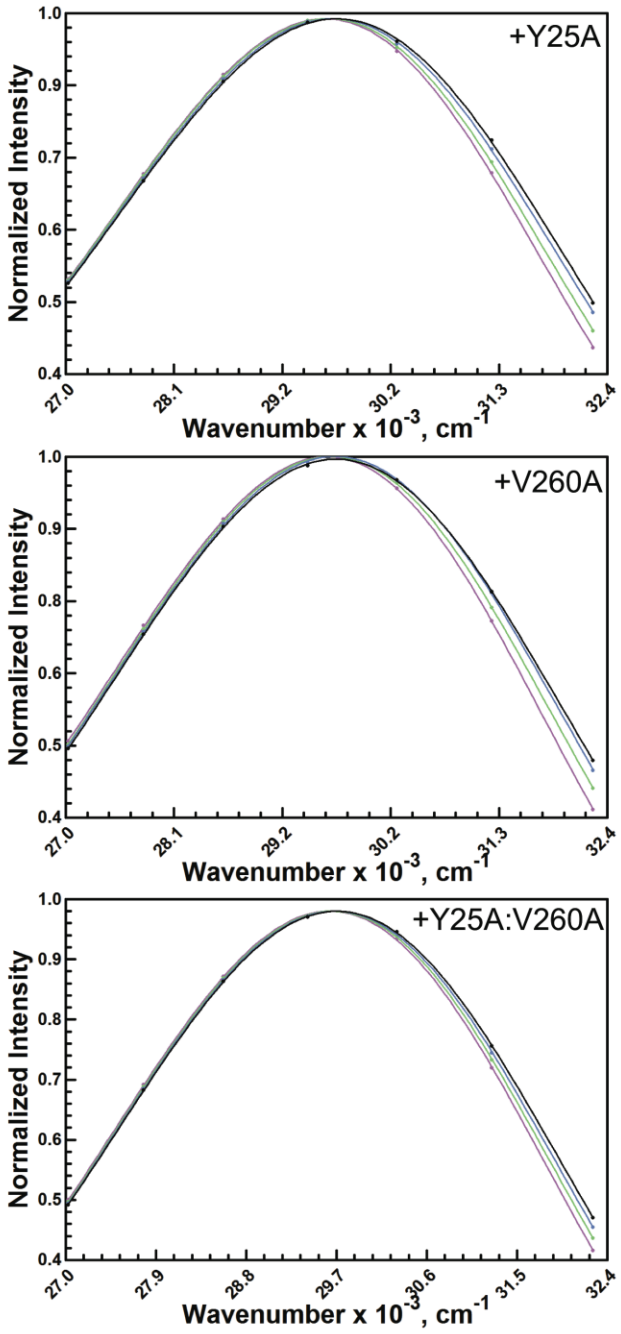

Figure S9. Temperature dependence of the Stern-Volmer plots for the W87in mutant series. Each additional mutation is indicated within the plot's inset. The temperatures shown are at 10ºC (black), 20ºC (blue), 30ºC (green), 40ºC (orange), 50ºC (red).

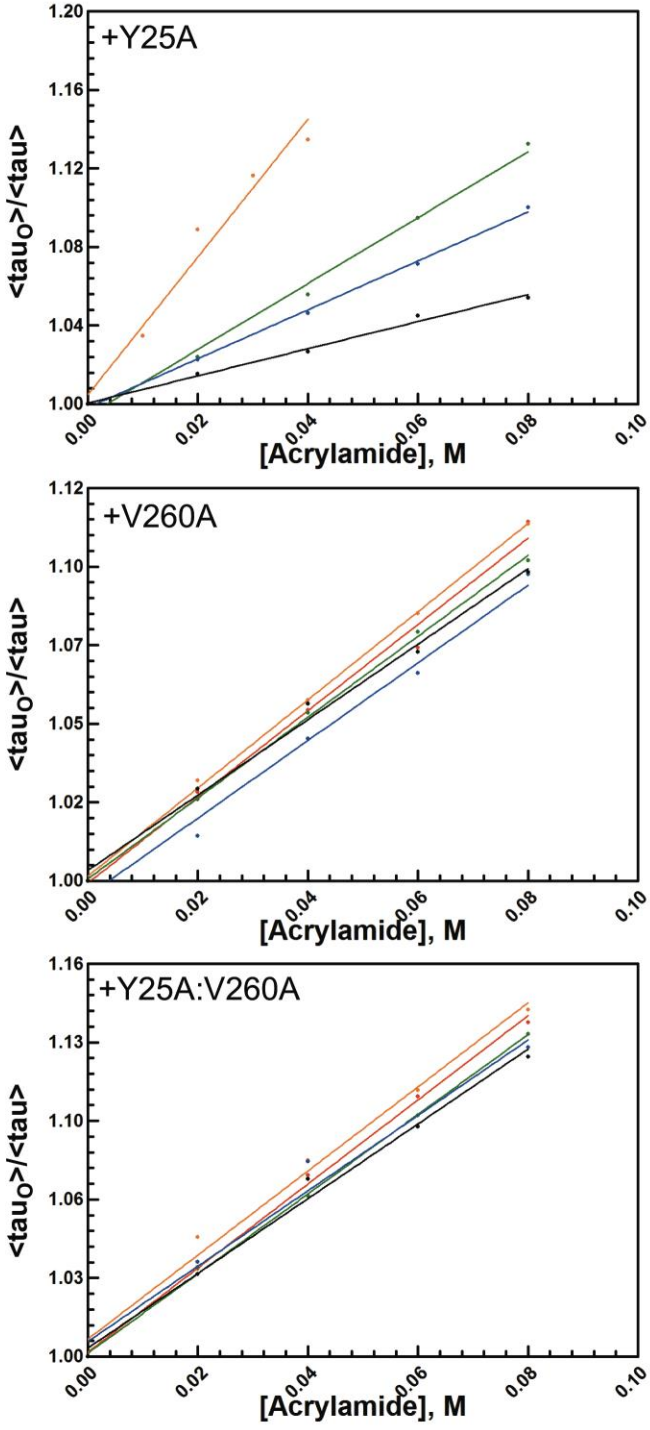

Figure S10. Temperature dependence of the Stern-Volmer plots for the W167in mutant series. Each additional mutation is indicated within the plot's inset. The temperatures shown are at 10ºC (black), 20ºC (blue), 30ºC (green), 40ºC (orange), 50ºC (red).