Supplementary Material for "The Cold Denatured State of the C-terminal Domain of Protein L9 Is Compact and Contains Both Native and Non-native Structure"

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Materials and Methods

Circular dichroism spectroscopy

CD detected thermal denaturation experiments were conducted as a function of pH between pH 4.8 and pH 8.0. The pH 4.7 thermal denaturation data was fit to a quadratic equation to obtain the unfolded state signal. The pH 8.0 curve was fit to the following equation to obtain thermodynamic parameters of the native state:

$$\theta(T) = \frac{(a_n + b_n T) + (a_d + b_d T) \exp(-\Delta G_u^{\circ}(T)/RT)}{1 + \exp(-\Delta G_u^{\circ}(T)/RT)} \quad (1)$$

where a_n, b_n, a_d and b_d are parameters which define the signals of the native state (N) and denatured state (D) at a given temperature. T_m is the heat induced unfolding midpoint temperature, $\Delta H^{o}(T_m)$ is the enthalpy change at T_m, and ΔC_p^{o} is the heat capacity change between the native and denatured states. The signal expected for a fraction of folded of 0.50 was estimated by taking the average of the native state signal and the unfolded state signal.

Stopped-flow fluorescence

Direct measurement of the exchange rate between the cold denatured state and the folded state at pH 5.7, 12^{o}C was performed by a fluorescence detected pH jump experiments. Protein was initially prepared at pH 8.0 and the pH was rapidly changed to pH 5.7 using an Applied Photophysics SX.18MV stopped flow instrument. The final

concentrations of proteins were approximately 100 μM. The folding and unfolding reactions were detected by monitoring the changes in the fluorescence of Tyr126. An excitation wavelength of 280 nm and a 305 nm cut-off filter were used for the measurements. The experiments were repeated 5 times to estimate the uncertainty. The experiments were conducted in 20 mM NaH_2PO_4 and 100 mM NaCl in H_2O.

Figure s1. Determination of the native state population at pH 5.7, 12 ^{o}C. CD detected thermal unfolding curves for I98A CTL9 monitored at different pH values. The red curve represents a quadratic fit to the pH 4.7 data and provides an experimental estimate of the unfolded state signal as a function of temperature since the fraction of folded is 0.0 under these conditions. The pH 8.0 data provides an estimate of the CD signal for the fully fold state as the fraction of folded is 1.0 at 25 ^{o}C, pH 8.0. The solid black line represents an extrapolation of the folded baseline. The green curve represents the signal expected for a fraction of folded of 0.50, i.e., the middle point of the transition. Analysis of the pH 5.7 curve (Δ) indicates a fraction of folded of 0.20 at 12 ^{o}C. (●) pH 8.0; (○), pH 6.6; (▼) pH 6.0; (Δ) pH 5.7; (■) pH 5.6; (□) pH 5.2; (◊) pH 4.7.

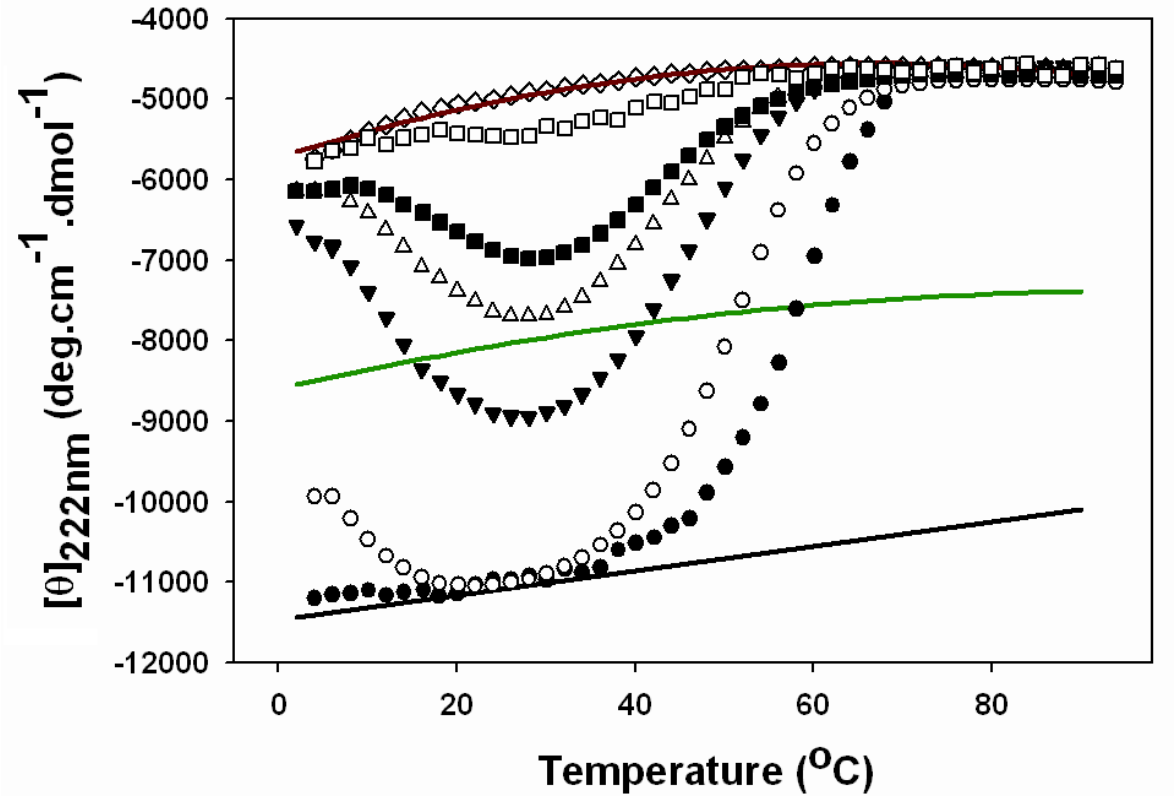


Figure s2. The time course of the change in fluorescence which is due to Tyr126. The curve is fit to a double exponential decay. A minor slow phase which corresponds to cis-trans proline isomerization in the unfolded state has a rate constant of 0.037 sec^{-1} *(1)*. The faster phase corresponds to folding and has a first order rate constant, $k_{obs} = k_{folding} + k_{unfolding}$, of 0.49 sec^{-1}. Folding and unfolding rates can be calculated using the estimated relative populations obtained from the data shown Figure S2. The calculated value for $k_{folding}$ is 8.8 x 10^{-2} sec^{-1} given the estimated folded population of 21% and the calculated value of $k_{unfolding}$ is 0.40 sec^{-1}. These rate constants correspond to a life time of 2.5 seconds (1/ $k_{folding}$) for the folded state and 11 seconds (1/ $k_{unfolding}$) for the cold denatured state.

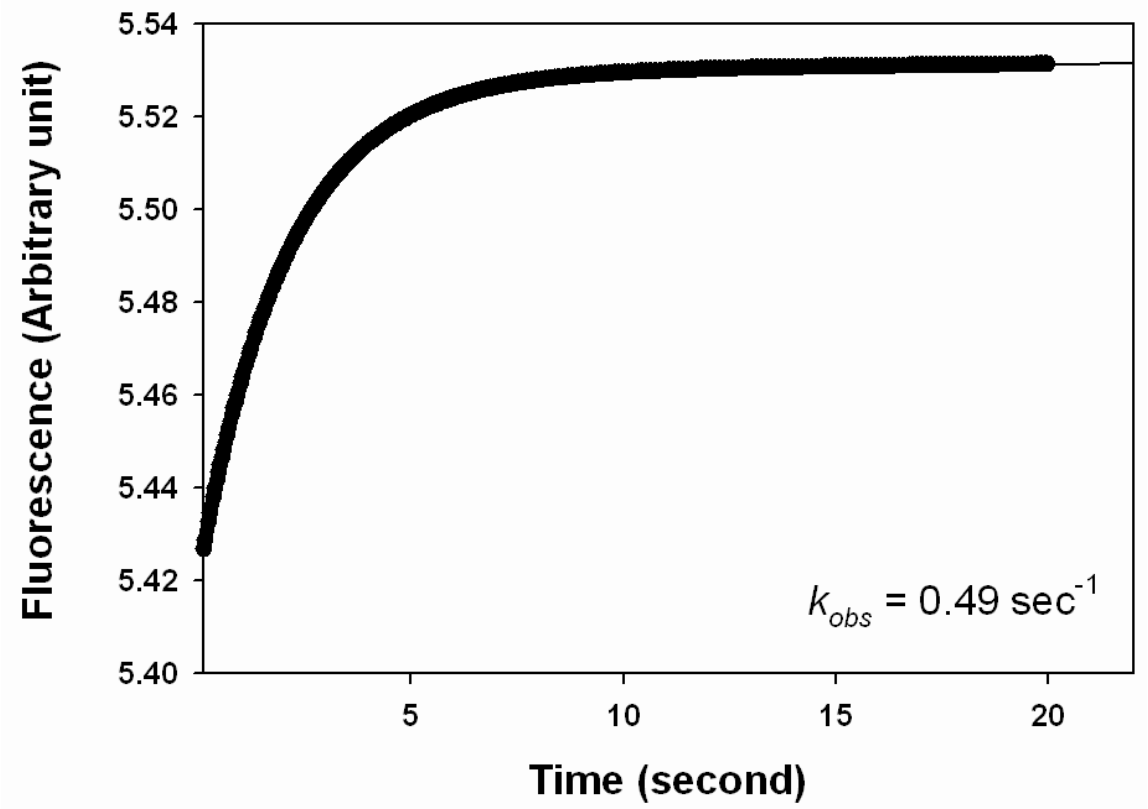
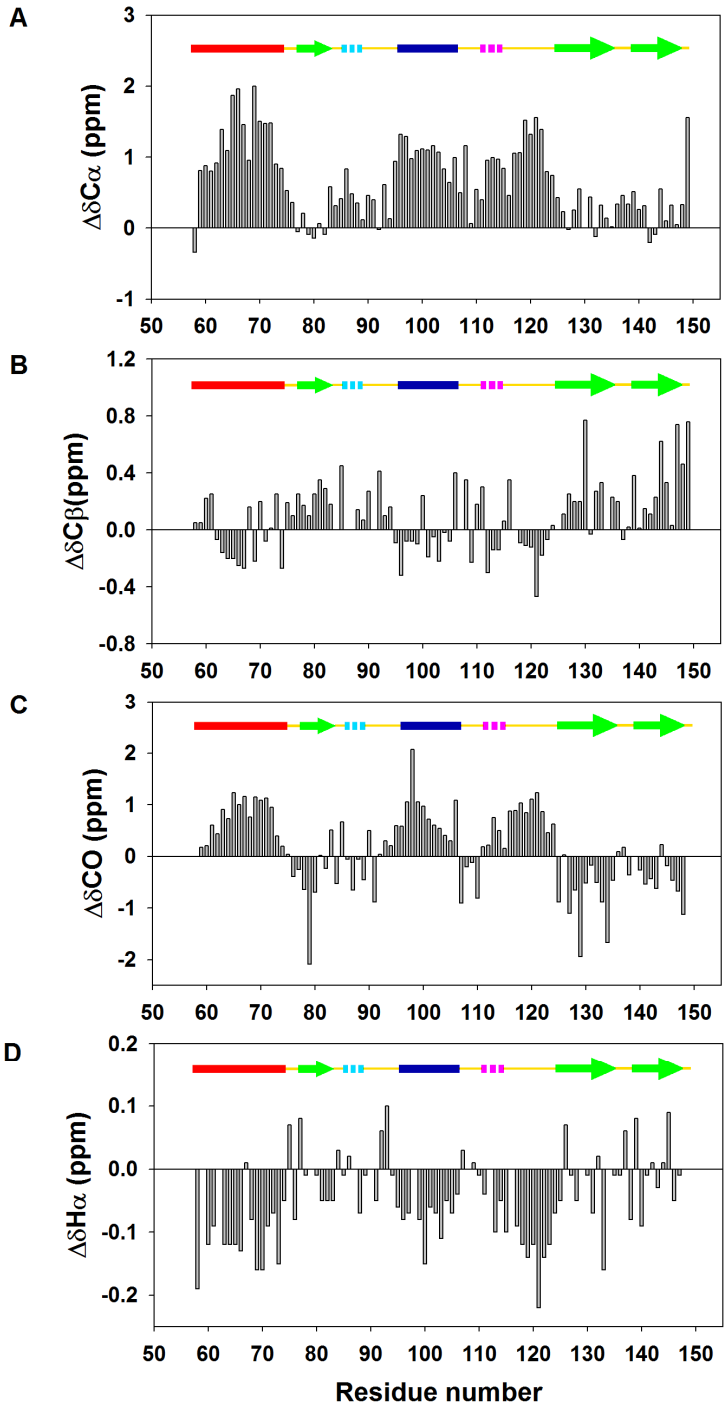


Figure s3. Plots of (A) $^{13}C_{\alpha}$ secondary shifts, (B) $^{13}C_{\beta}$ secondary shifts, (C) ^{13}CO secondary shifts and (D) $^{1}H_{\alpha}$ secondary shifts for the cold denatured state of I98A CTL9. Assignments were obtained at pH 5.7, 12 ^{o}C . A schematic diagram of the elements of secondary structure of the native state of CTL9 is shown at the top of each panel.



References

1. Sato, S.; Raleigh, D. P. *J. Mol. Biol.* **2002**, *318*, 571-582.