# How Quickly Can a β-Hairpin Fold From Its Transition State?

## Supporting Information

*Beatrice N. Markiewicz,[1] Lijiang Yang,[2,3] Robert M. Culik,[4] Yi Qin Gao,[2,3,*] Feng Gai[1,*]*

[1]Department of Chemistry and [4]Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania 19104, United States

[2] Beijing National Laboratory for Molecular Sciences, College of Chemistry and Molecular Engineering, and [3]Biodynamic Optical Imaging Center; Peking University, Beijing, 100871, China

### Global Fit of CD Thermal Denaturation Curves to a Two State Model

To quantitatively assess the thermal stabilities of the Trpzip4 cross-linked and uncross-linked variants, we globally fit the CD unfolding curves to the following two state model:

$$\theta(i,T) = \frac{\theta_{\mathrm{U}}(i,T) + K_{\mathrm{eq}}(i,T)\theta_{\mathrm{F}}(i,T)}{1 + K_{\mathrm{eq}}(i,T)} \tag{1}$$

$$K_{\mathrm{eq}}(i,T) = \exp(-\Delta G(i,T)/\mathrm{R}T) \tag{2}$$

$$\Delta G(i,T) = \Delta H_{\mathrm{m}}(i) + \Delta C_{\mathrm{p}}(T - T_{\mathrm{m}}) - T[\Delta S_{\mathrm{m}}(i) + \Delta C_{\mathrm{p}} \ln(\frac{T}{T_{\mathrm{m}}})] \tag{3}$$

where $\theta(i,T)$ is the temperature-dependent mean residue ellipticity of peptide $i$, $K_{\mathrm{eq}}(i,T)$ is the corresponding equilibrium constant for unfolding, $T_{\mathrm{m}} = \Delta H_{\mathrm{m}}(i)/\Delta S_{\mathrm{m}}(i)$ is the thermal melting temperature, $\Delta H_{\mathrm{m}}(i)$ and $\Delta S_{\mathrm{m}}(i)$ are the enthalpy and entropy changes at $T_{\mathrm{m}}$, and $\Delta C_{\mathrm{p}}$ is the heat

capacity change. In addition, the folded and unfolded CD baselines, $\theta_F(i,T)$ and $\theta_U(i,T)$, respectively, are assumed to be linear functions of temperature, as follows:

$$\theta_U(i,T) = c + dT \tag{4}$$

$$\theta_F(i,T) = a_i + bT \tag{5}$$

where $b$, $c$, and $d$ were treated as global fitting parameters and $a_i$ was peptide dependent and treated as a local fitting parameter.

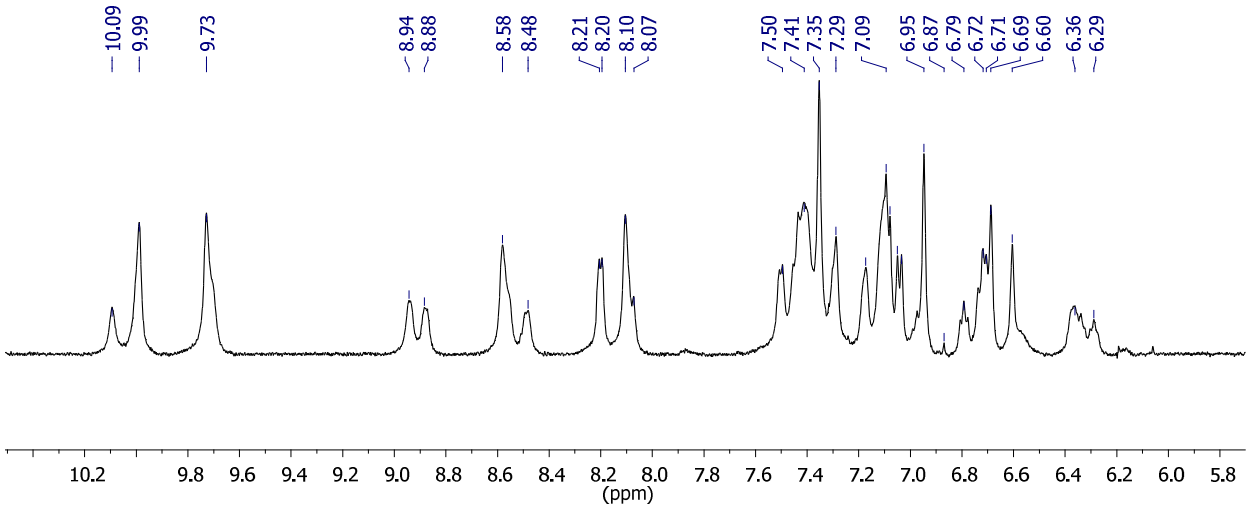


**Figure S1.** $^1$H-NMR spectrum of TZ4-T-CL in the amide proton region, collected at room temperature on a Bruker AVIII (cryo500) (Bruker, Billerica, MA) NMR spectrometer. The NMR sample was prepared by dissolving lyophilized peptide in $H_2O/D_2O$ (90%/10%) to a final concentration of 1 mM (pH ~3).

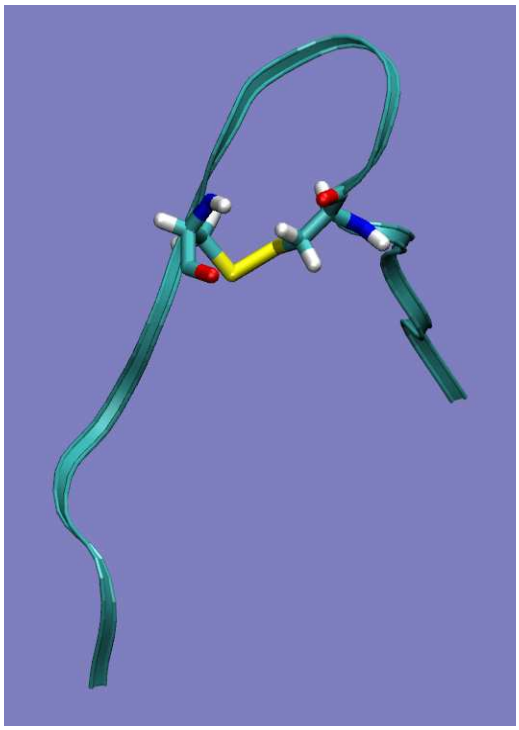

**Figure S2.** A representative structure of the $U_A$-like unfolded state obtained from MD simulations.

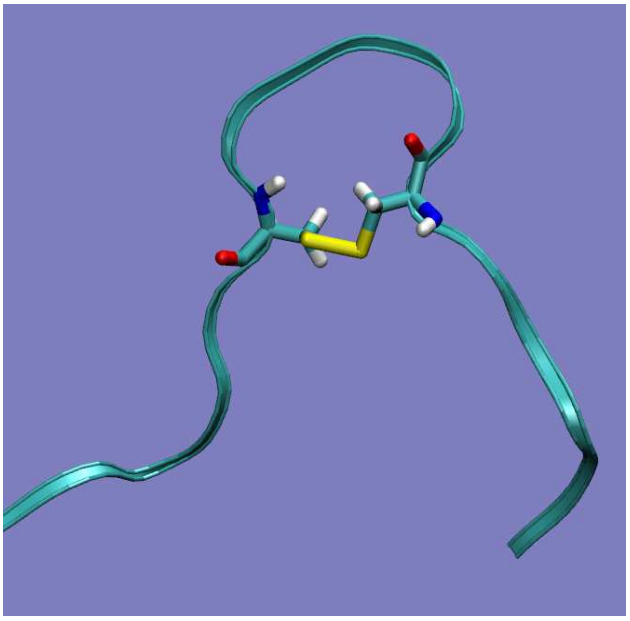

**Figure S3.** A representative structure of the $U_B$-like unfolded state obtained from MD simulations.