Supporting Information

The distributed, ensemble-convergent MD simulations reported here were performed on a large number of structures of $T\beta_9$ ($200 \leq n \leq 400$). Molecular construction and MD simulations were carried out using the CHARMM[1] suite of programs with the all-atom CHARMM22[2] force-field and analyzed with the aid of VMD.[3] The starting-point structure was obtained from the 2D ^{1}H NMR experimental data deposited under 1HJ0 ID in the RCSB Protein Data Bank (www.pdb.org).[4] The initial PDB structure was energy-minimized for 12000 steps *in vacuo* and then heated to T = 300 K and pre-equilibrated for 100 ps. The structure was equilibrated for 1 ns at T = 300 K and, from the latter equilibration step, n = 400 random configurations of $T\beta_9$ were obtained and further equilibrated for 1 μs to represent the (isolated) macromolecular ensemble at T_0 = 300 K. In order to assess the ensemble-averaged temperature-jump dynamics of $T\beta_9$, the above-mentioned equilibrated macromolecular ensemble was used as a starting point for three sets of n = 400 independent heating trajectories representing the ΔT = 300 K, 600 K, and 900 K temperature jumps in UED simulations. For each of these trajectories, the starting-point macromolecular configuration was heated to the final temperature, $T = T_0 + \Delta T$, within 1 ps and allowed to evolve for up to 1 μs to obtain the unfolding statistics.

In order to assess the fraction of intact native contacts as well as both local and global structural changes throughout the ensemble, two complementary types of data were collected as a function of time. First, for all sets of independent trajectories, the fraction of each native (helical) contact remaining intact at time t was calculated as follows. The fraction of intact hydrogen bonds was obtained for every native-contact pair and further averaged over the n = 200 (400) independent trajectories to obtain the average decay of each native contact as a function of time. A hydrogen bond was defined to be 100 % intact if the distance between the donated proton and the nitrogen or oxygen atom (the hydrogen acceptor) was less than 1.8 Å and the angle defined by NH of the donor residue and O of the acceptor residue was at least 120°. In addition, the smoothness of the

transition between fully intact and a fully broken hydrogen bonds was enforced using an exponential attenuation of the bond strength such that the hydrogen bond would be 1/e-fold intact at a distance of 2.5 Å. These criteria are consistent with established conventions for geometry-based hydrogen bond determination,[5] and it should be noted that the fast process of native-contact disruption renders the results thus obtained to be insensitive to variance in the threshold values used.

Second, the ensemble-averaged radial distribution functions, $\langle f(r, t)\rangle_n$ and $\langle f_B(r, t)\rangle_n$, were calculated at time t by employing a locally-modified version of the UEDANA code using an artificial damping factor of $k = 0.02$ Å^2 to compensate for the unwanted oscillations induced by a finite data range ($s_{max} < \infty$).[6] Root-mean-square (RMS) amplitudes of thermal vibrations, $l_{ij} = u_{ij}$, were estimated using empirical equations[7] at $T_0 = 300$ K and further extrapolated to elevated (final) temperatures, $T = T_0 + \Delta T$, using Equation (4) of Ref. 6. From the above variations of $\langle f(r, t)\rangle_n$ and $\langle f_B(r, t)\rangle_n$ with time, as obtained for a variety of temperature jumps using large ($n = 200$) macromolecular ensembles of Tβ$_9$ generated during the course of ensemble-convergent MD simulations, the temporal profiles of ensemble-averaged, root-mean-square radii of gyration, $(\langle R_g^2(t)\rangle_n)^{1/2}$, characteristic of the ensembles were calculated as described here and in Ref. 8. We note that $\langle R_g^2(t)\rangle_n$ can also be obtained directly from the Cartesian coordinate sets of the ensembles.[8]

References

1 (a) B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, M. Karplus, *J. Comp. Chem*. **1983**, *4*, 187-217.
(b) A. D. MacKerell, Jr., B. Brooks, C. L. Brooks, III, L. Nilsson, B. Roux, Y. Won, M. Karplus, in *The Encyclopedia of Computational Chemistry*, Vol. 1, Eds. P. v. R. Schleyer, John Wiley & Sons, Chichester, **1998**, pp. 271-277.

2 A. D. MacKerell, Jr., D. Bashford, M. Bellott, R. L. Dunbrack, Jr., J. D. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, T. Ngo, D. T. Nguyen, B. Prodhom, W. E. Reiher, III, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiórkiewicz-Kuczera, D. Yin, M. Karplus, *J. Phys. Chem. B* **1998**, *102*, 3586-3616.

3 W. Humphrey, A. Dalke, K. Schulten, *J. Molec. Graphics* **1996**, *14*, 33-38.

4 R. Stoll, W. Voelter, T. A. Holak, *Biopolymers* **1997**, *41*, 623-634.

5 I. Y. Torshin, I. T. Weber, R. W. Harrison, *Protein Eng*. **2002**, *15*, 359-363.

6 D. Shorokhov, S. T. Park, A. H. Zewail, *Chem. Phys. Chem*. **2005**, *6*, 2228-2250.

7 (a) V. S. Mastryukov, S. J. Cyvin, *J. Mol. Struct*. **1975**, *29*, 16 -25.
(b) E. L. Osina, V. S. Mastryukov, L. V. Vilkov, S. J. Cyvin, *J. Struct. Chem*. **1975**, *16*, 977-978.
(c) S. J. Cyvin, V. S. Mastryukov, *J. Mol. Struct*. **1976**, *30*, 333-337.
(d) V. S. Mastryukov, E. L. Osina, *J. Struct. Chem*. **1976**, *17*, 147-148.
(e) V. S. Mastryukov, *J. Struct. Chem*. **1976**, *17*, 69 -73; (f) V. S. Mastryukov, E. L. Osina, L. V. Vilkov, S. J. Cyvin, *J. Struct. Chem*. **1976**, *17*, 64-68.

8 M. M. Lin, D. Shorokhov, A. H. Zewail, *Chem. Phys. Lett*. **2006**, *420*, 1-7.