Molecular Mechanism Behind the Fast Folding/Unfolding Transitions of Villin Headpiece Subdomain: Hierarchy and Heterogeneity

Toshifumi Mori* and Shinji Saito*

Institute for Molecular Science, Myodaiji, Okazaki, Aichi, 444-8585, Japan, and School of Physical Sciences, The Graduate University for Advanced Studies, Okazaki, Aichi 444-8585, Japan

E-mail: mori@ims.ac.jp; shinji@ims.ac.jp

Phone: +81-564-55-7305; +81-564-55-7300

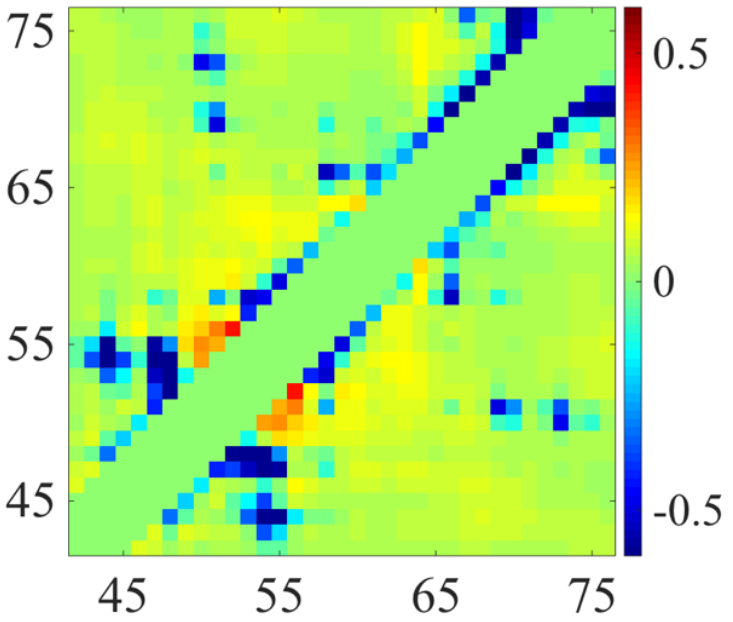

Figure S1: Contact map of the unfolded state, given as a difference from the native state ensemble (Fig. 1(b)).

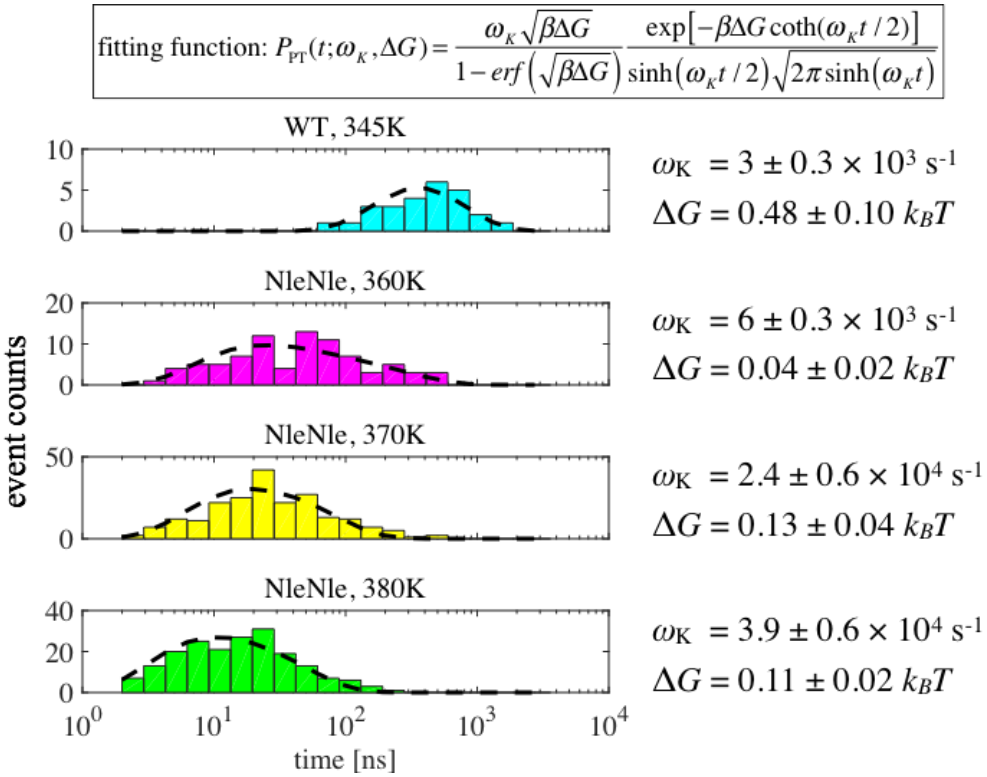

Figure S2: Fitting curves of the TPT distributions in Fig. 3 using the transit across harmonic barrier model at the high-barrier limit.[1,2] The functional form used for fitting the histograms and the obtained parameters are also given in the figure.

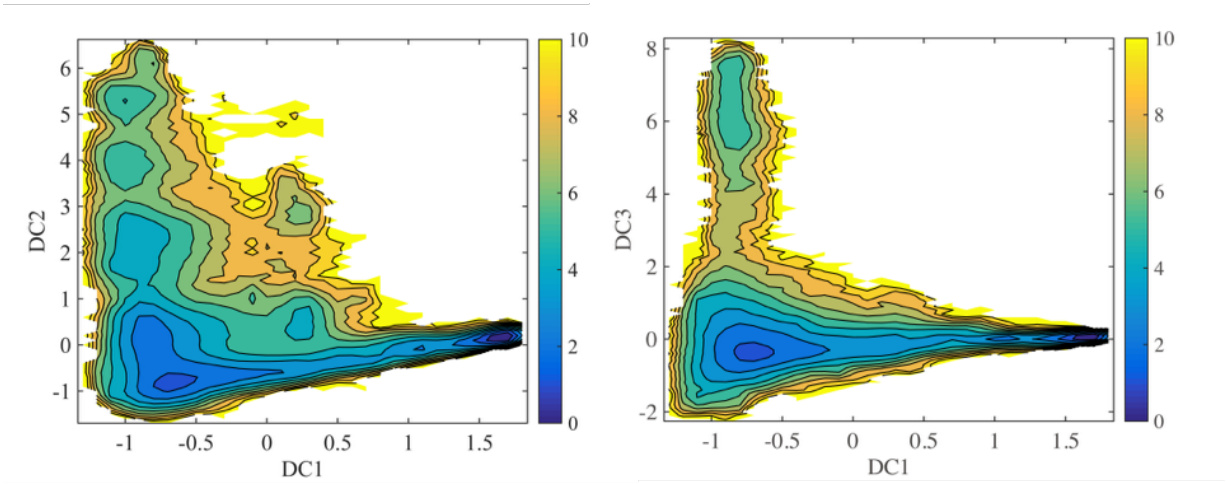

Figure S3: Two dimensional free energy surface as a function of DC1 and DC2 (left) and DC3 (right) for WT at 345 K. DC2 and DC3 can be characterized by the interaction between Leu61-Trp64 and Thr54-Trp64, respectively, and represent the conformational heterogeneity within the unfolded state.

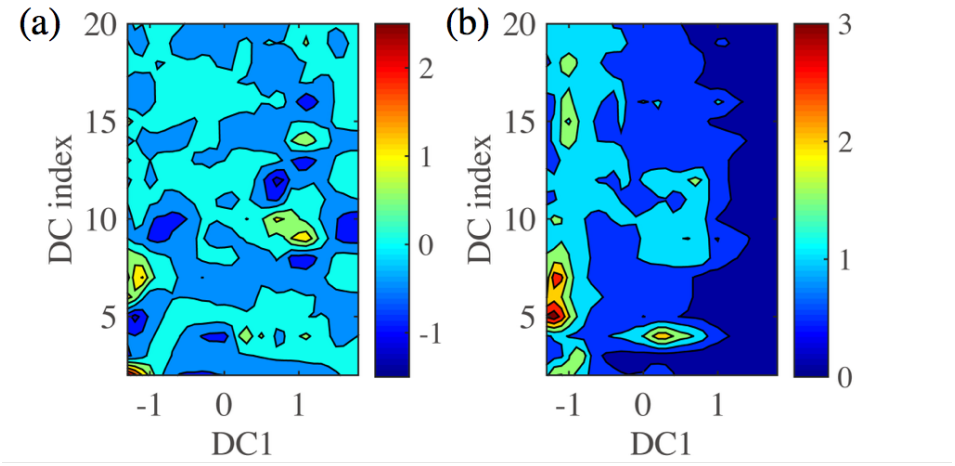

Figure S4: The (a) mean and (b) standard deviation of the 2nd to 20th DC modes along DC1 for WT at 345 K. Note that the mean and the standard deviation indicate the coupling and flexibility of the modes along DC1, respectively.

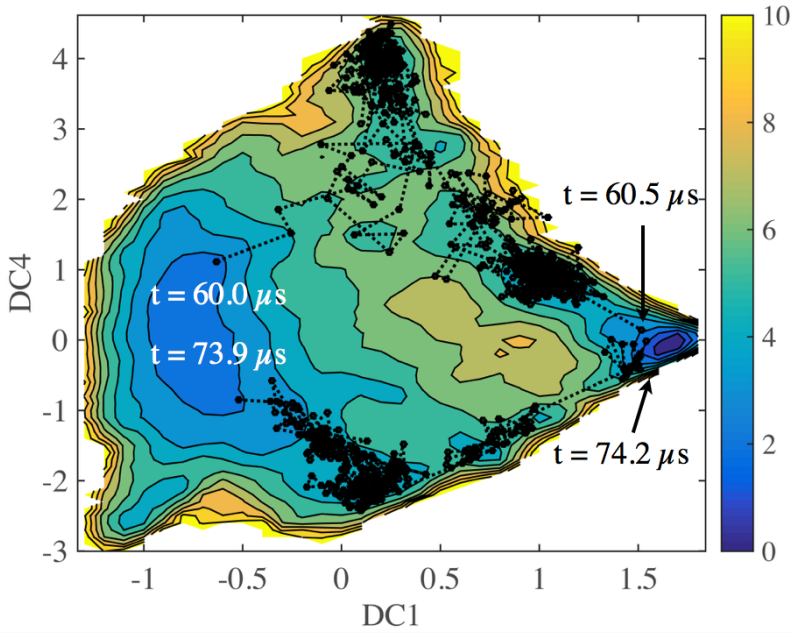

Figure S5: Two representative transition trajectories that proceeds via the two major pathways from the WT trajectory at 345 K. The underlying two dimensional free energy is identical to that in Fig. 4. The initial and final times of each trajectory segment are also shown in white and black, respectively.

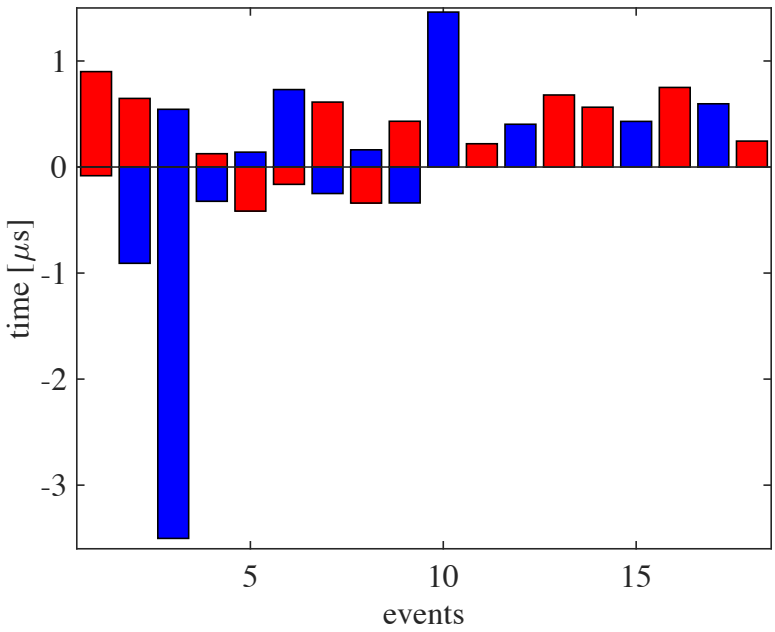

Figure S6: Lengths of each transition events found in the WT trajectory. The events are classified into transitions via α_3 melting and α_1-α_2 deforming pathways simply by the average of DC4 over each transition, i.e. $\langle \mathrm{DC4} \rangle_i > 0$ and $\langle \mathrm{DC4} \rangle_i \leq 0$, respectively, and are given in positive and negative times. Here, $\langle \cdots \rangle_i$ denotes the time average over the trajectory for transition event i. Blue and red bars represent the fold and unfolding transitions, respectively.

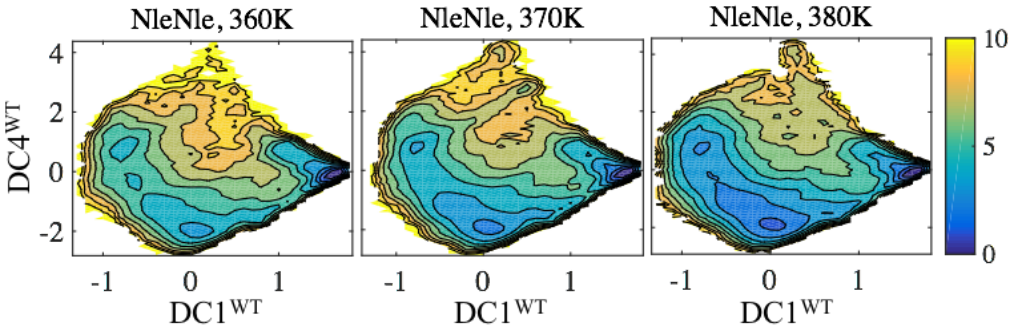

Figure S7: Two dimensional free energy surfaces as a function of DC1 and DC4 for NleNle. Left, center, and right figures are for 360, 370, and 380 K, respectively. Note that for easy comparison to Fig. 4, the coordinates are optimized for the WT trajectory, which is indicated by the superscript "WT".

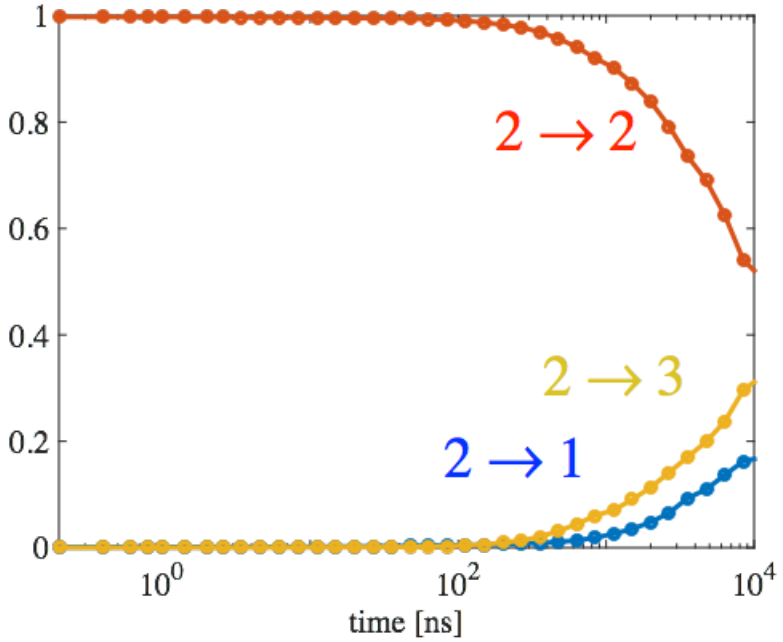

Figure S8: Ratio of population transferred from the native state of WT. The initial population is selected such that DC1 > 1.55 as well as $0.3 \geq E_{47} < 0.7$. Blue, red, and yellow colors show the ratio of population transferred to state 1, 2, and 3 from the native state (which is also state 2), respectively.

References

(1) Chaudhury, S.; Makarov, D. E. A harmonic transition state approximation for the duration of reactive events in complex molecular rearrangements. *J. Chem. Phys.* **2010**, *133*, 034118.

(2) Neupane, K.; Foster, D. A. N.; Dee, D. R.; Yu, H.; Wang, F.; Woodside, M. T. Direct observation of transition paths during the folding of proteins and nucleic acids. *Science* **2016**, *352*, 239–242.