Single Molecule Force Spectroscopy Reveals the Context Dependent Folding Pathway of the C-terminal Fragment of Top7

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Electronic Supplementary Information

Refolding of Top7 occurs in a narrow range of forces

The refolding of Top7 occurs in a narrow range of forces, which is in sharp contrast to the broad distribution of the unfolding forces. Refolding occurring at low forces over a narrow force range of a few pN appears to be a very generic phenomenon, and have been observed in a number of other proteins.[1-7] Recent studies proposed that in the force spectroscopy experiments, the collapsing from a more extended polypeptide conformation to a much more compact globular structure may play an important role in the folding of proteins against a stretching force.[3, 8]This behavior is generic in protein folding against a stretching force, and may represent a possible mechanism for the common narrow distribution of the refolding forces observed in different proteins.

References

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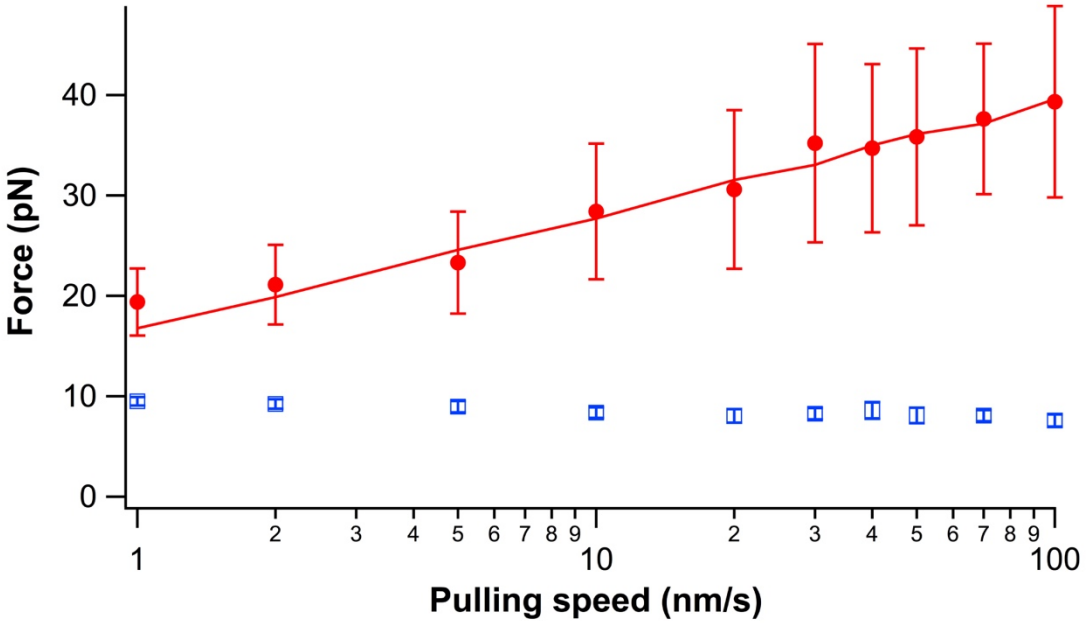

Figure S1. Pulling speed dependence of the unfolding (red) and refolding (blue) forces of Top7. Solid lines are Monte Carlo simulation results using a Δx_u of 0.7 nm and an α_0 of 0.001 s^{-1} for unfolding.

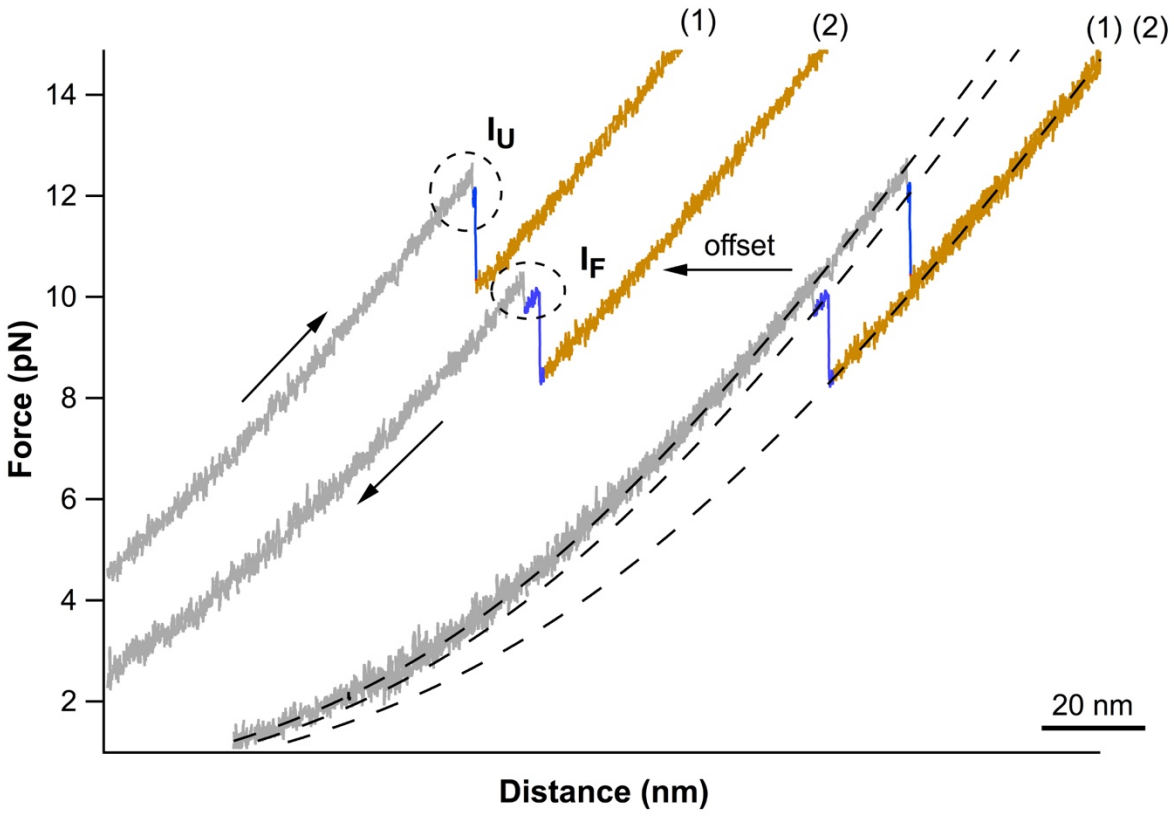

Figure S2. The folding and unfolding intermediates (I_U and I_F) have similar contour length increment ΔLc. The dotted lines are pseudo-WLC fits to the force-distance curves. The folded state is colored in grey, intermediate in blue and unfolded in brown. To visualize the intermediate more clearly, the offset stretching (curve 1) and relaxation (curve 2) curves are shown on the left of the graph.

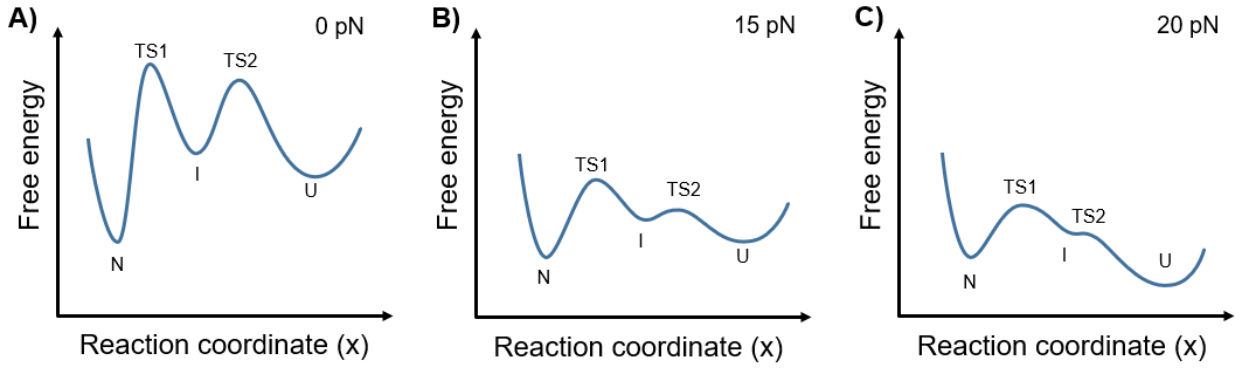

Figure S3. Schematics of the unfolding energy landscape of Top7 under an applied force of 0 pN (A), 15 pN (B) and 20 pN (C). N: native state, I: intermediate, U: unfolded state, TS: transition state.

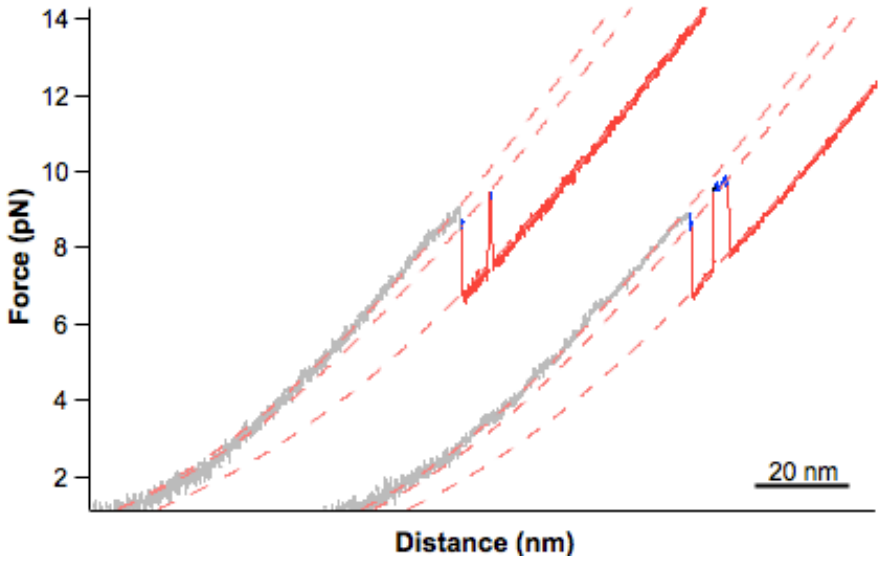

Figure S4. The folding intermediate of Top7 was observed in rare cases to unfold back to unfolded state during relaxing in constant speed experiments.

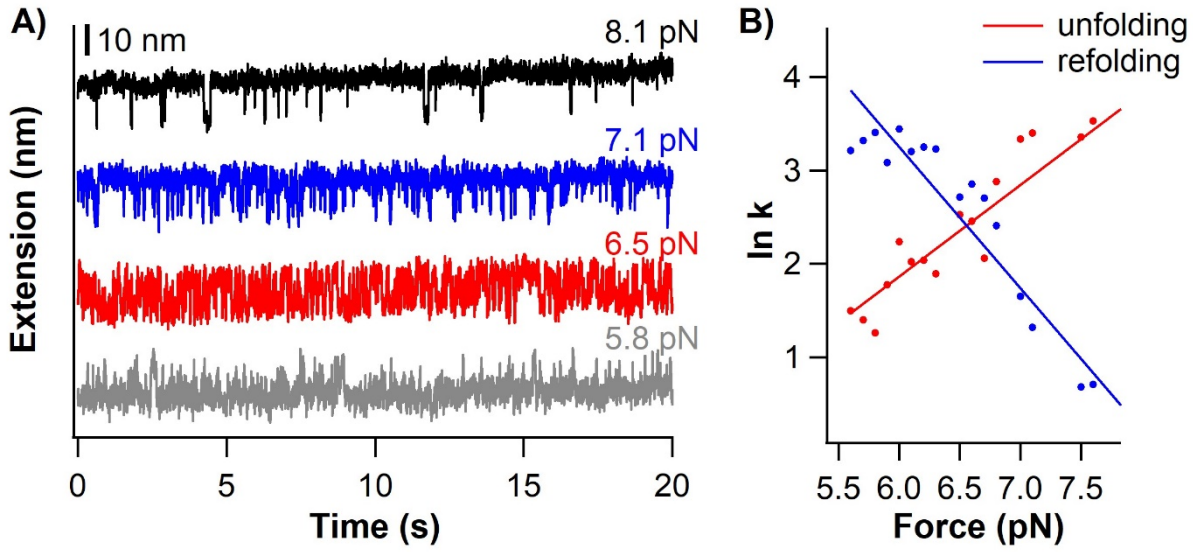

Figure S5. A) CFr fluctuated between native and unfolded state in constant force experiment. B) Force-dependent folding-unfolding rates of CFr derived from the constant force experiment. Δx_u and Δx_f are determined to be 4.03 ± 0.46 nm and 6.21 ± 0.53 nm, and α_0 and β_0 are determined to be 0.02 ± 0.01 s^{-1} and $2.3*10^5 \pm 1.9 * 10^5$ s^{-1}, which are very close to those derived from constant speed experiment.

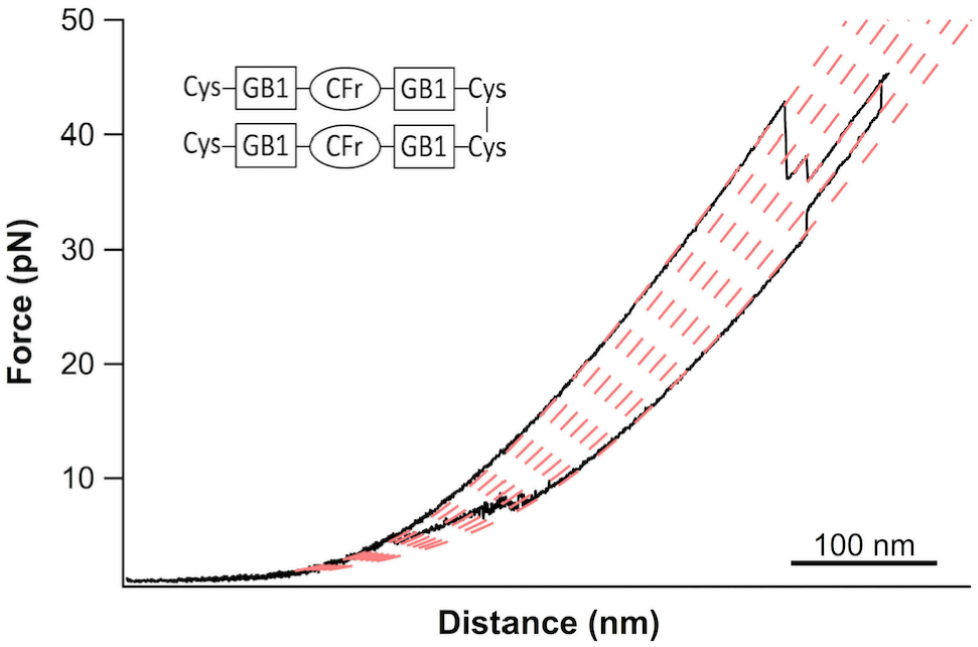

Figure S6. Force-distance curves of dimerized GB1-CFr-GB1 under a pulling speed of 50 nm/s. Dashed lines are pseudo-WLC fitting. Three individual GB1 unfolding events can be clearly observed, and the other unfolding event with ΔL_c of ~54 nm probably arises from the dissociation and unfolding of CFr dimer and GB1 domain. Inset shows the schematic dimer structure of GB1-CFr-GB1 formed by cysteine crosslinking.

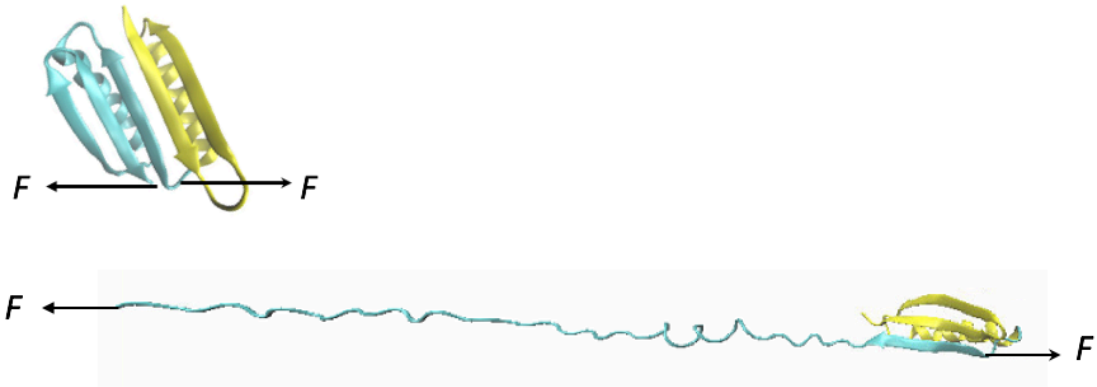

Figure S7. SMD simulations of the unfolding CTh. The N terminal half (NTh) was observed to retain its secondary structure during the simulation trajectory after the CTh has unraveled.