Mechanically Untying a Protein Slipknot: Multiple Pathways Revealed by Force Spectroscopy and Steered Molecular Dynamics Simulations

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Supporting Information

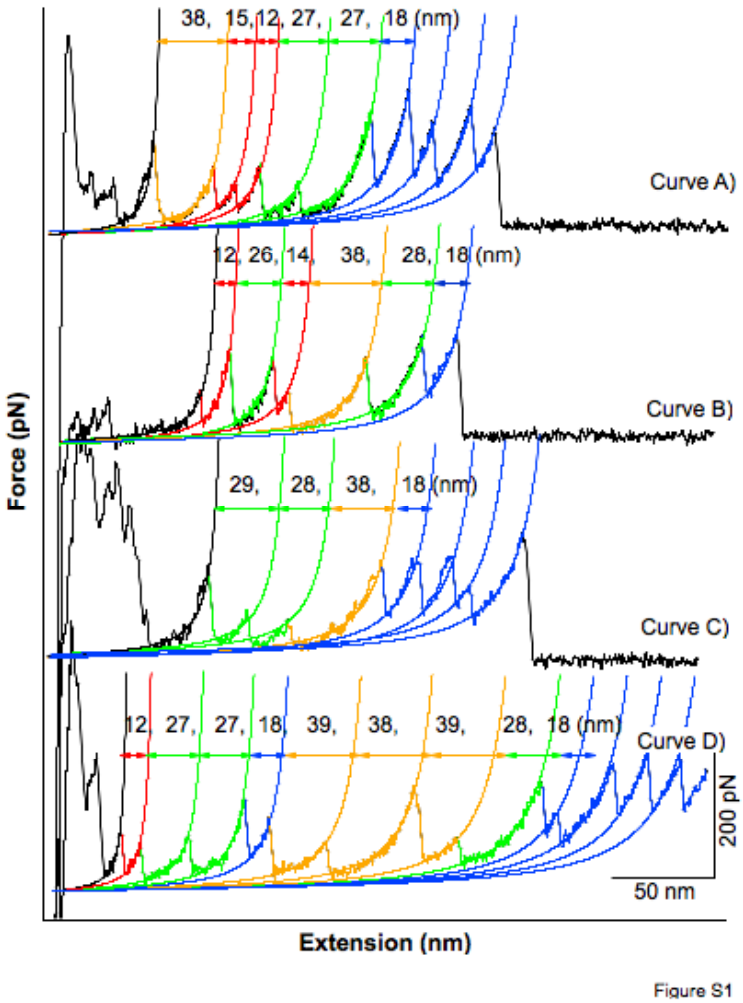

Figure S1. Force-extension curves of $(GB1\text{-}AFV3\text{-}109)_n$. The mechanical unfolding of GB1 fingerprint domains is colored in blue and characterized by a ΔL_C of 18 nm. The unfolding events of AFV3-109 are colored according to their unfolding pathways: two-state unfolding is colored in orange, N-I in red and I-U in green, respectively.

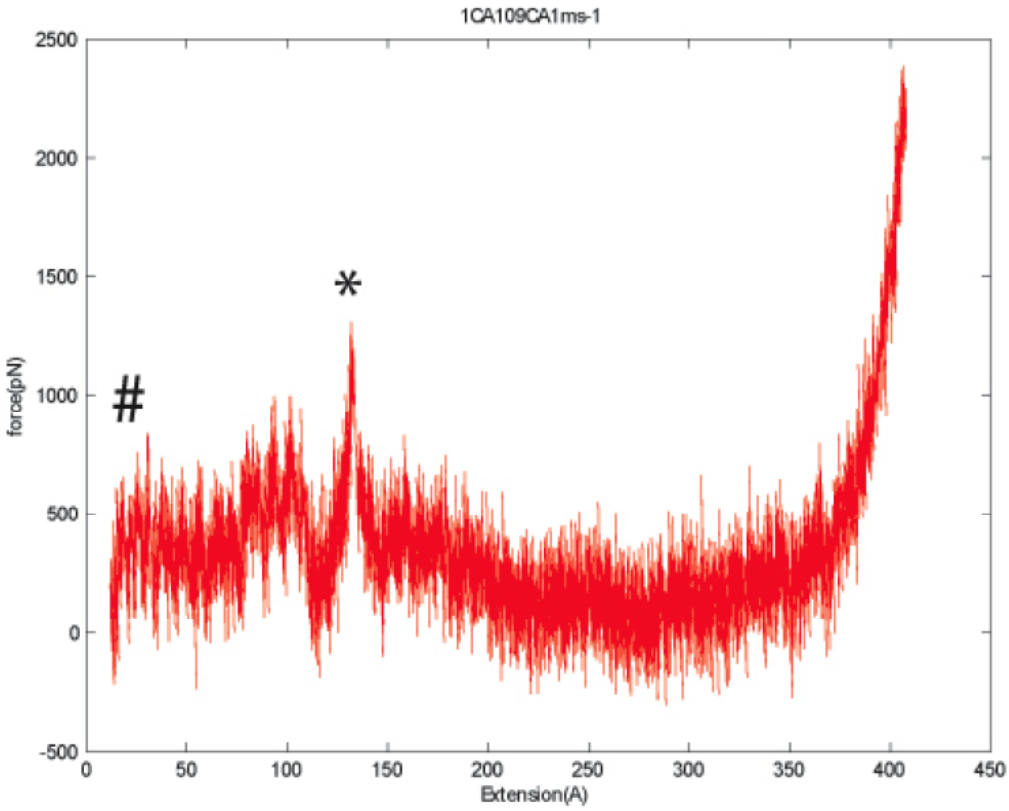

Figure S2. A typical unfolding trajectory without a clear initial unfolding force peak indicated by #. The unfolding force peak due to the rupture of the mechanical unfolding intermediate state I is indicated by *.

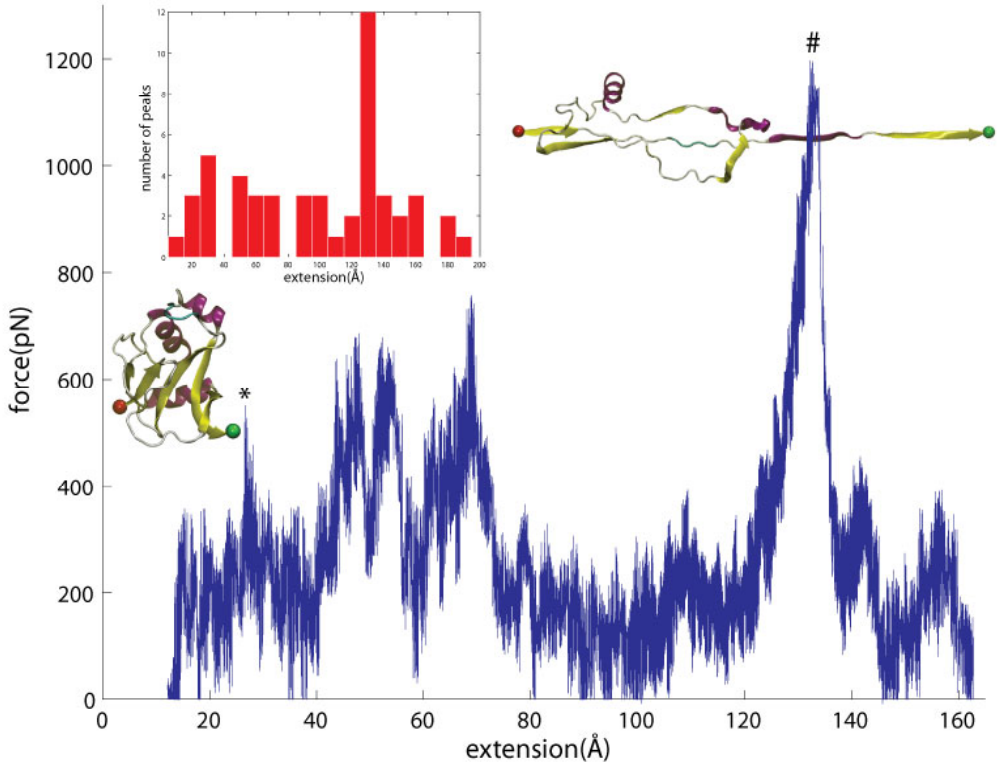

Figure S3. Non-specific interactions can lead to extra unfolding force peaks in SMD simulations. Inset: histogram of the occurrences of the unfolding force peaks with different contour length increments. For unfolding trajectories following this type of pathway, the intermediate state I always occurs. However, the initial peak occurs at a much lower frequency, and some extra peaks were observed due to friction when pulling the threaded loop out of the knotting loop.

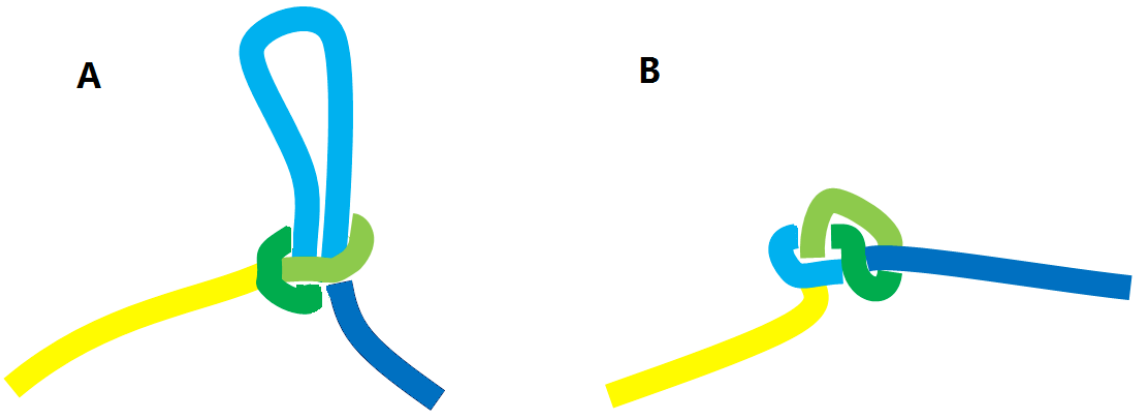

Figure S4. Comparison between the tightened slipknot of AFV3-109 (A) and tightened figure-eight knot in Phytochrome (B). The tightened figure-eight knot is more complex and bigger than the tightened slipknot. The tightened figure-eight knot contains 17 residues and is ~6.2 nm in size.

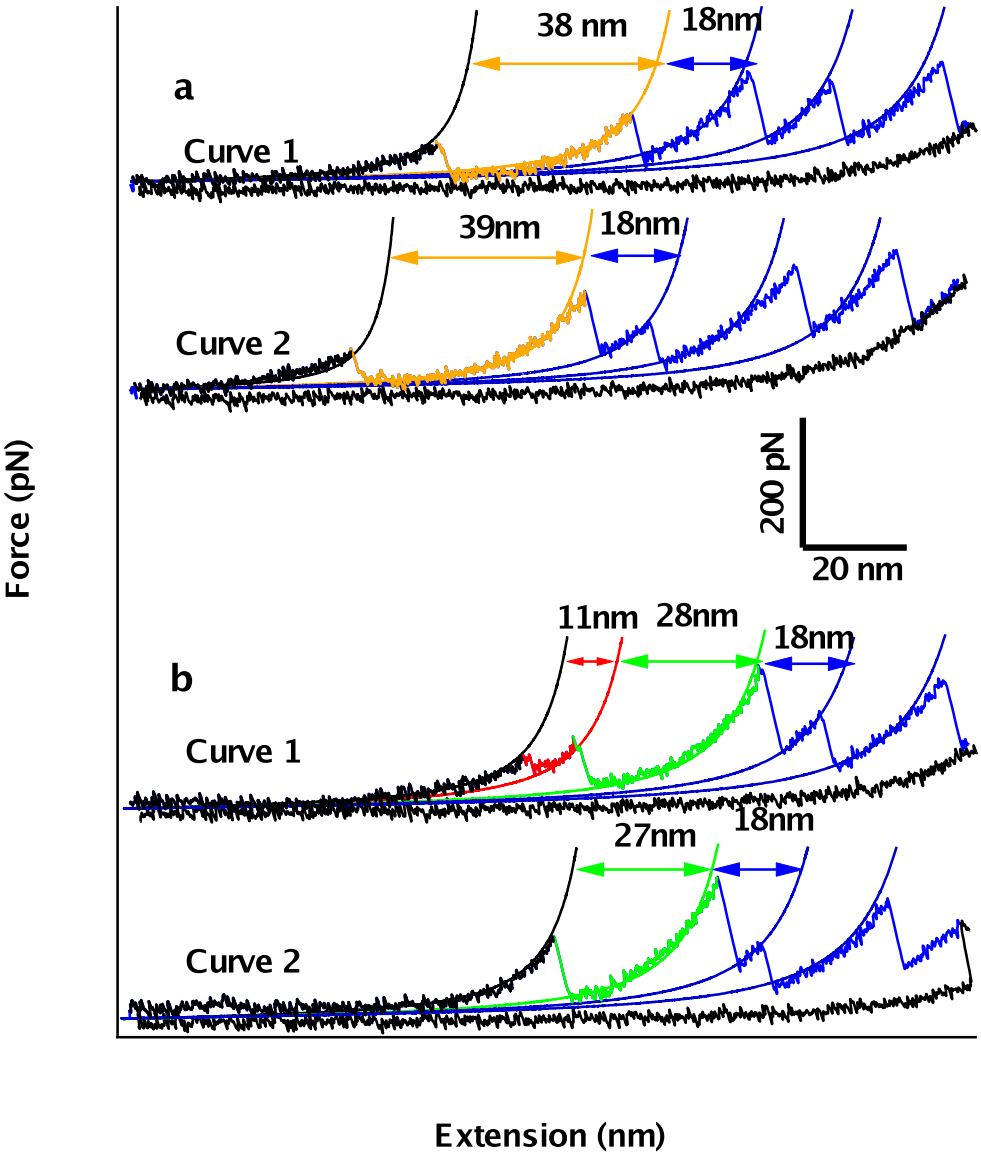

Figure S5. Unfolded AFV3-109 can refold back to its native state (a) as well as unfolding intermediate state prior to its complete folding (b). The mechanical unfolding of GB1 fingerprint domains is colored in blue and characterized by a ΔL_C of 18 nm. The unfolding events of AFV3-109 are colored according to their unfolding pathways: two-state unfolding is colored in orange, N-I in red and I-U in green, respectively.