

Mechanically Tightening, Untying and Retying a Protein Trefoil

Knot by Single Molecule Force Spectroscopy

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Electronic Supplementary Information

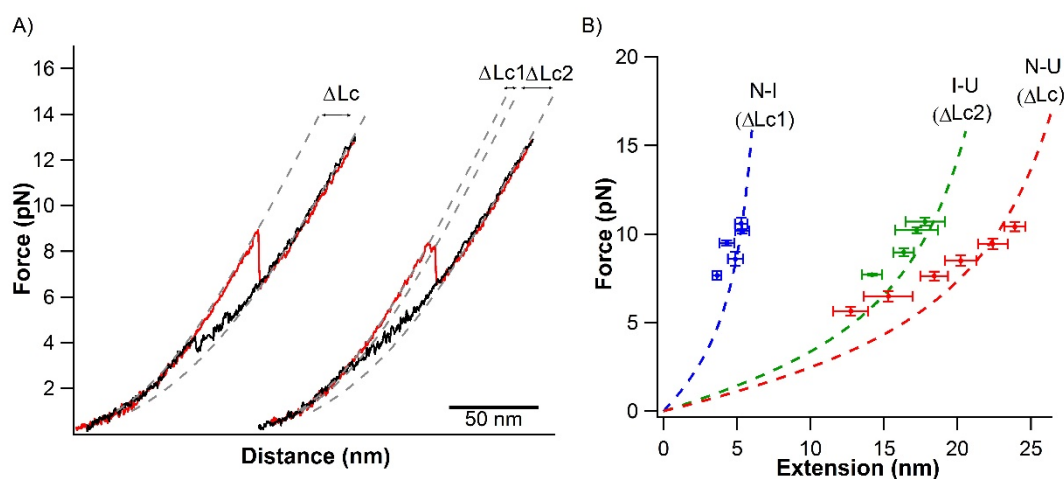


Figure S1. Mechanical unfolding and folding of TrmD Δ helix_{9,161}. A) Representative force-distance curves showing the unfolding and folding signatures were identical to those of TrmD_{9,246}. Both two-state and three-state unfolding were observed for protein unfolding. Upon relaxation, the tightened trefoil knot followed a quasi-two state pathway to the native state. B) Force-extension relationship of TrmD Δ helix_{9,161}. The WLC fits measured a persistence length of 0.8 nm and $\Delta Lc1$ of 8.7 ± 0.7 nm, $\Delta Lc2$ of 29.7 ± 1.6 nm and ΔLc of 37.5 ± 2.4 nm, respectively.

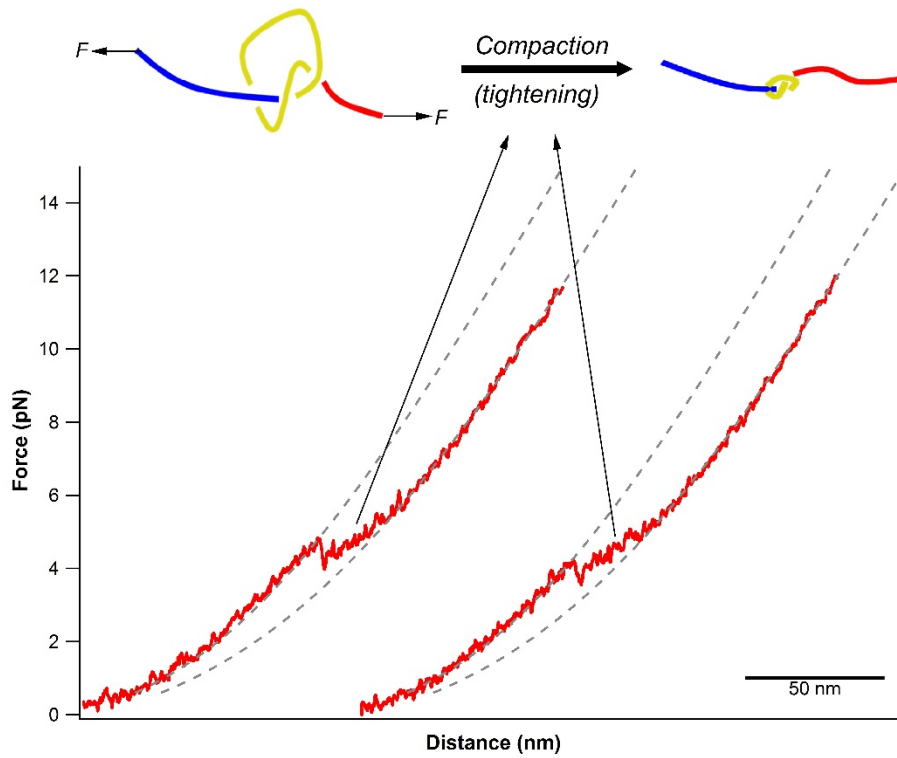


Figure S2. Representative force-distance curves showed the mechanical unfolding of TrmD_{9,246} at low forces. A clear deviation from a typical WLC behavior of an unfolded polypeptide chain can be observed at the early stage. Further stretching leads to the polypeptide lengthening until the force-distance curve returns to follow the WLC behavior. This polypeptide lengthening process corresponds to the compaction and tightening of the knot structure within the protein, which is shown in the schematics on the top.

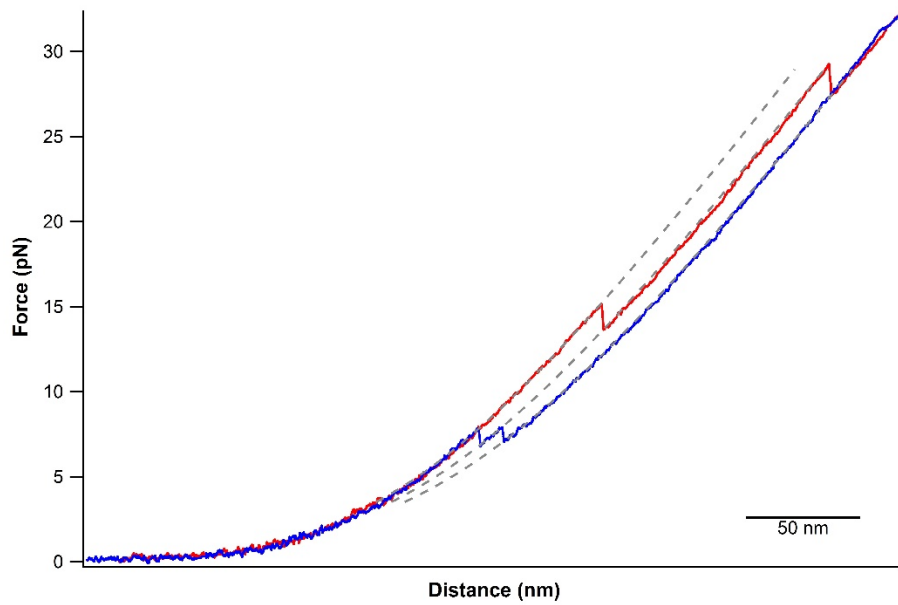


Figure S3. Mechanical unfolding and folding of two NuG2 protein domains at a pulling speed of 50 nm/s. The unfolded NuG2 domains follow the typical WLC behavior of a unfolded polypeptide chain.

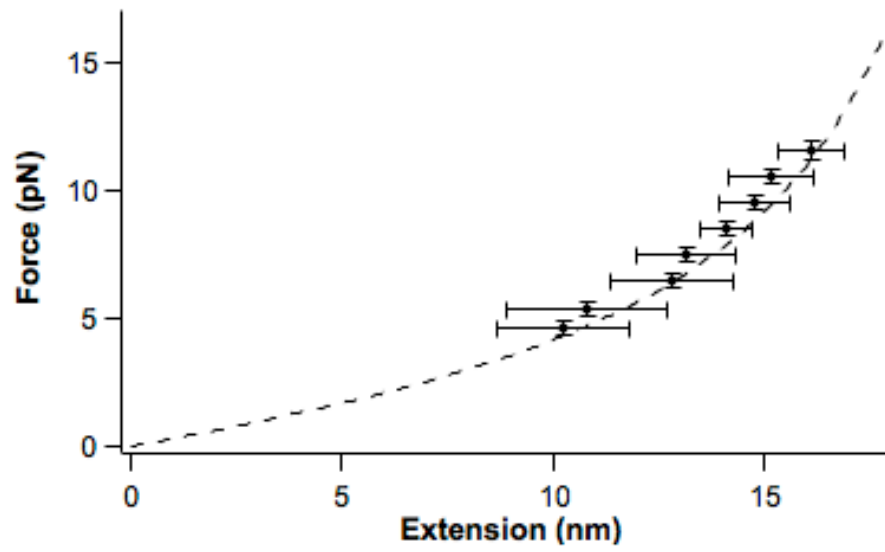


Figure S4. Force-extension relationship of TrmD Δ helix_{45,128}. WLC fits yielded the persistence length of 0.8 nm and Δ Lc of 25.6 ± 0.7 nm.

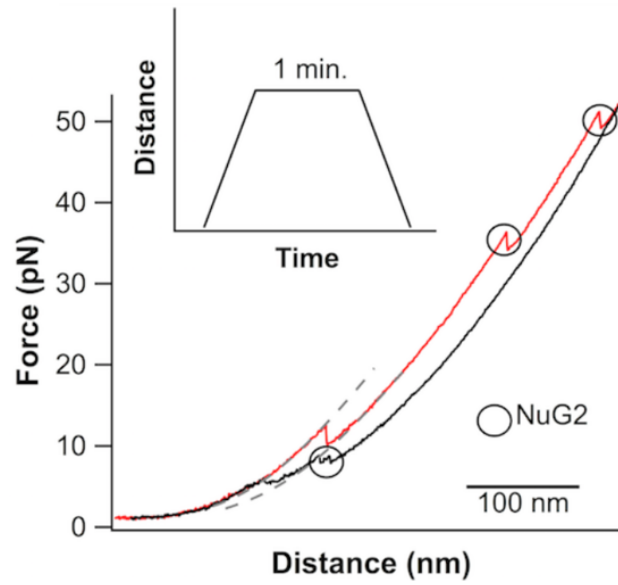


Figure S5. High tension does not inhibit the folding of TrmD_{9, 246}. After TrmD_{9, 246} and NuG2 were unfolded (in red), the protein was stretched to a high force (~50 pN) and held at this force for 1 min. During the subsequent relaxation, TrmD_{9, 246} was observed to fold into its native state successfully. The inset shows the experimental protocol.

Table S1. Average unfolding/folding forces and kinetic parameters for TrmD_{9, 246} and TrmD Δ helix_{45,128}

		F_u (pN)	F_f (pN)	α_0 (s ⁻¹)	Δx_u (nm)	β_0 (s ⁻¹)	Δx_f (nm)	ΔLc (nm)
TrmD _{9, 246}	N-U	8.0±1.8 (n=371)	4.5±0.7 (n=543)	$(3.8\pm0.5)\times 10^{-3}$	2.9±0.3	$(3.3\pm0.3)\times 10^2$	4.4±0.3	36.8±1.8
	N-I	9.5±1.3 (n=172)	N/A	$(3.6\pm0.6)\times 10^{-3}$	2.9±0.4	N/D [#]	N/D	9.8±0.4
	I-U	8.9±1.3 (n=172)	N/A	$(3.6\pm0.3)\times 10^1$	0.5±0.1	N/D	N/D	28.9±1.0
TrmD Δ helix _{45,128}	N-U	8.2±2.3 (n=142)	4.8±0.6 (n=142)	$(2.5\pm2.2)\times 10^{-1}$	0.7±0.4	$(2.5\pm0.4)\times 10^3$	5.3±1.1	25.6±0.7

N/D: not determined

Table S2. Number of molecules, unfolding and folding events of TrmD and its mutants

	# of molecules	# of unfolding events	# of folding events
TrmD _{9,246}	13	543	543
TrmD Δ helix _{9,161}	5	240	240
TrmD _{45,128}	5	76	76
TrmD Δ helix _{45,128}	9	142	142

The full sequence of the engineered TrmD_{9,246}-(NuG2)₂-Cys

MRGSHHHHHHGSMWIGIISLC**PEMFRAITDYGVTGRAVKNGLLSIQSWSPR**
DFTHDRHRTVDDRPYGGGPGMLMMVQPLRDAIHAAKAAAGEGAKVIYL
SPQGRKLDQAGVSELATNQKLILVSGRYEGIDERVIQTEIDEEWSIGDYVL
SGGELPAMTLIDSVSRFIPGVLGHEASATEDSFAEGLLDSPHYTRPEVLEG
MEVPPVLLSGNHAEIRRWRLKQSLGRTWLRRPELLENLALTEEQARLLA
EFKTEHAQRSMDTYKLVIVLNGTTFTYTTEAVDAATAEKVFKQYANDNGVDGEW
TYDDATKTFTVTERSMDTYKLVIVLNGTTFTYTTEAVDAATAEKVFKQYANDNGVD
*GEW***TYDDATKTFTVTERS****GGTK**C

The sequence in bold encodes TrmD_{9,246} and the sequence in italic corresponds to NuG2 domains. Cysteine residues designed as the stretching sites are underlined.