Supplementary Online Material for:
The Mechanochemistry Of a Structural Zinc Finger

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Materials and Methods

Protein engineering and purification

The genes codifying DnaJΔ_{107} and its mutants were amplified by PCR using the appropriate primers containing the restriction sites for BamHI, BglII and KpnI. $(I27)_2DnaJ_{\Delta 107}(I27)_2$, $(Zf_1Zf_2\text{-}I27)_4$ and $(I27)_2DnaJ_{\Delta 107(C161/164/197/200S)}(I27)_2$ were designed using I27 as fingerprint. The rest of the constructs, in which it is neccessary to unambiguously identify Domain I, were cloned using ProteinL as molecular marker. All polyproteins were cloned into the pQE80L (Qiagen) expression vector and transformed into the BLR (DE3) *Escherichia coli* expression strain. Cells were grown in LB broth supplemented with 100 μg/mL ampicillin at 37^0C. After reaching an OD_{600} of ~0.6 cultures were induced with 1 mM Isopropyl β-D-1-thiogalactopyranoside and incubated overnight at 25^0C. Cells were disrupted with a French Press and the polyproteins from the lysate were purified by metal affinity chromatography on Talon resin (Takara, Clontech) followed by gel-filtration using a Superdex 200 10/300 GL column (GE Biosciences). Proteins were stored in PBS buffer at 4 ºC.

APPY peptide interaction.

The polyprotein $(PL)_2DnaJ_{\Delta 107}(PL)_2$ (30 μM) was incubated overnight at 4 ºC in the presence of APPY at different concentrations, ranging from 5 μM to 150 μM, in PBS pH 7.4 buffer. The samples were centrifuged prior to the AFM experiments.

Single Molecule Force Spectroscopy experiments

Single molecule experiments were conducted at room temperature using both a home-made set-up described elsewhere[1] and a commercial Luigs and Neumann force spectrometer[2]. Each protein sample was prepared by depositing 1-5 μL of protein (at a concentration of 0.5-1.5 mg/mL) onto a freshly evaporated gold cover slide. All the experiments were carried out using PBS buffer at pH 7.4. Each cantilever (Si_3N_4 Bruker MLCT-AUHW) was individually calibrated using the equipartition theorem, yielding a typical spring constant of ~15 pN/nm. Single proteins were picked out from the surface with a constant force of 1500-2000 pN to promote the nonspecific adhesions of the proteins to the cantilever surface. In the force extension experiments, the pulling speed was set at 400 nm/s.

Data Analysis

All data were recorded and analysed using custom software written in Igor Pro 6.32 (WaveMetrics, Lake Oswego, OR).

Figure SI1

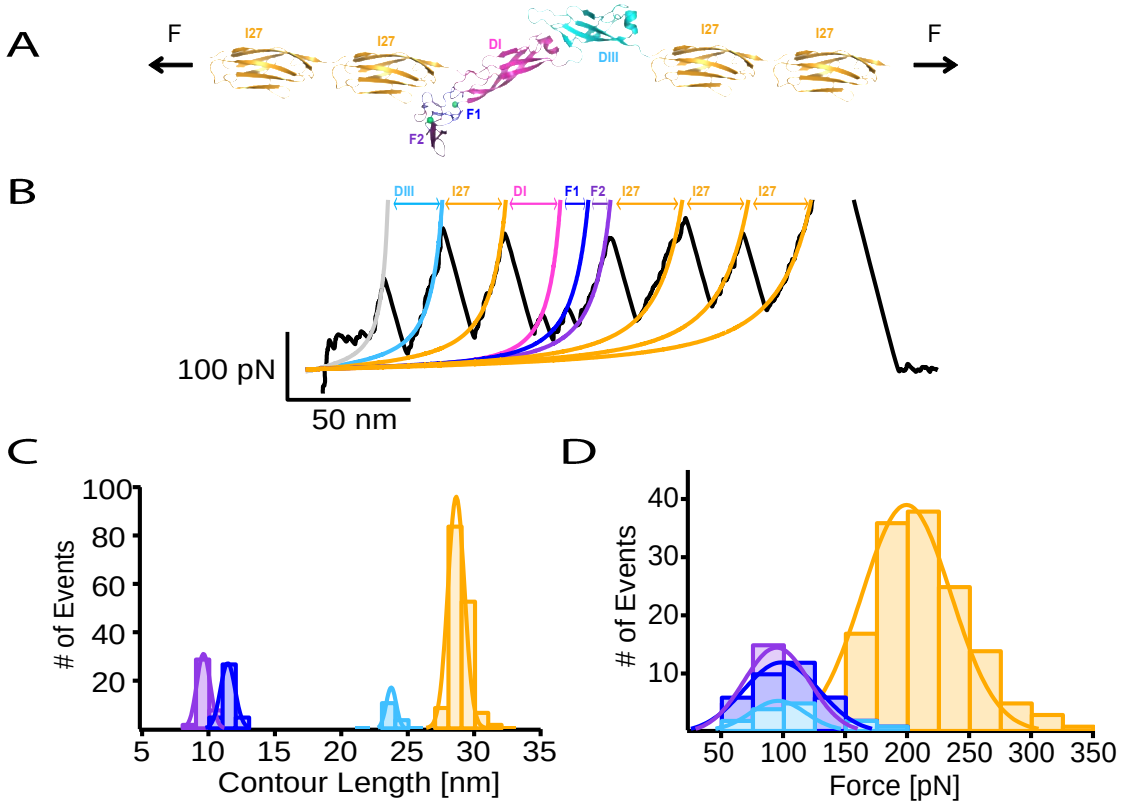

Figure SI1. The sequential mechanical unfolding of DnaJ does not follow a hierarchy in the mechanical stability. A) Scheme of the engineered $(I27)_2DnaJ_{\Delta 107}(I27)_2$ polyprotein. B) Typical force-extension trajectory corresponding to the mechanical unfolding of the $(I27)_2DnaJ_{\Delta 107}(I27)_2$ polyprotein. C, D) Histogram corresponding to the contour length increase (C) and force (D) hallmarking each individual unfolding event. Unfolding of Domain III requires a force of 86.3 ± 22 pN (n= 14) and occurs concomitant to an increase in contour length of ΔL_c = 23.4 ± 0.4 nm, (cyan). The unfolding of the labile Zinc-Fingers occurs only after the unfolding of Domain I; the first zinc motif (blue) is characterized by ΔL_c = 11 ± 0.5 nm (n= 37), requiring a force of 90.5 ± 31 pN, while the second zinc finger requires 85.7 ± 26 pN (n = 39) and elicits ΔL_c = 9.1 ± 0.5 nm (purple).

Finally the I27 fingerprints unfold at 194.7 ± 36 pN (n= 143) with a concomitant ΔL_c = 28.3 ± 0.6 nm (yellow).

Figure SI2

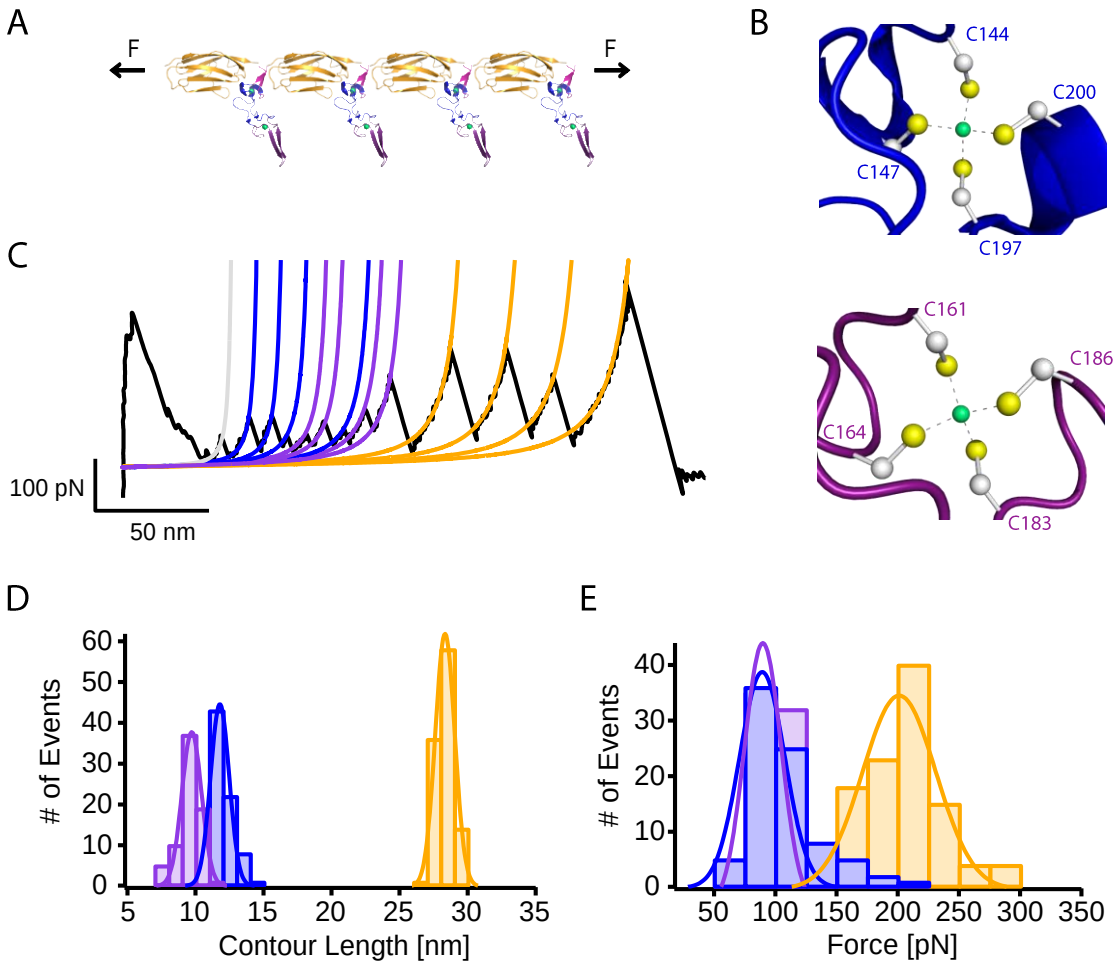

Figure SI2. Mechanical unfolding of the zinc fingers occurs at surprisingly low forces. A) Scheme of the engineered $(Zf_1Zf_2\text{-}I27)_4$ polyprotein, PDB: 1exk. B) Detail of the Zn^{2+} coordination sphere for the first (top, blue) and second (bottom, purple) Zn-Finger motifs. C) Typical force-extension trajectory corresponding to the mechanical unfolding of the $(Zf_1Zf_2\text{-}I27)_4$ polyprotein. D) The rupture of each individual zinc finger is hallmarked by an increase in contour length of ΔL_c = 11.2 ± 0.7 nm (zinc finger 1, blue) and ΔL_c = 9.1 ± 0.7 nm (zinc finger 2, purple), requiring forces of 84.1 ± 18 pN (n = 82) and 86.6 ± 16 pN (n = 72),

respectively, (E). The I27 marker unfolds at 191.5 ± 29 pN (n = 109), with a concomitant ΔL_c = 27.8 ± 0.7 nm (yellow).

Figure SI3

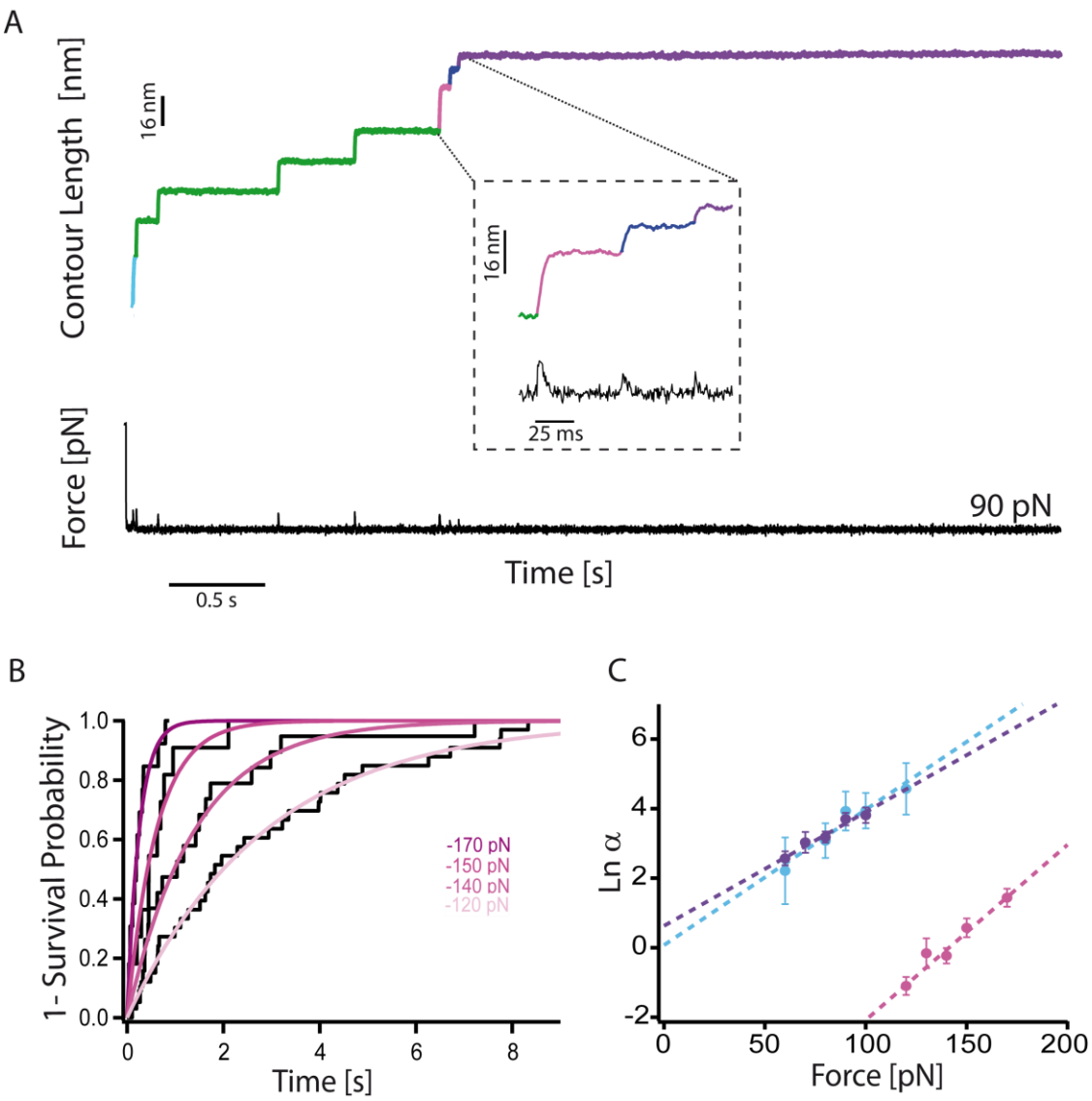

Figure SI3. The sequential mechanical unfolding of DnaJ is observed in Force-Clamp experiments. A) Typical force-clamp trajectory corresponding to the mechanical unfolding of the $(PL)_2DnaJ_{\Delta 107}(PL)_2$polyprotein at a constant force of 90 pN. In a typical unfolding trajectory, Domain III (cyan) unfolds first in a step of ~ 21 nm, followed by the unfolding of the PL monomers (green), marked by the ~16 nm steps. The mechanically resistant

Domain I (pink) occurs at a longer time, concomitant to a step increase in length of ~24 nm. The unfolding of Domain I triggers the fast (~15 ms) rupture of the first (blue) and second (purple) zinc fingers, fingerprinted by an increase in length of ~ 9 nm and ~ 7 nm, respectively. B) The cumulative probability of Domain I unfolding at a constant force can be captured as a first approximation by a single exponential, yielding the unfolding rate at this particular probed force. Varying the stretching force in the range spanning from 120 pN to 170 pN allows us to measure the dependency of the unfolding rate $\alpha(F)$ with the pulling force. C) Semi-logarithm plot of the unfolding rate constant as a function of the pulling force, $\alpha(F)$, corresponding to the unfolding of Domain I (pink), Domain III (cyan) and Finger 2 (purple). Fitting the Bell model $\alpha(F) = \alpha_0 \exp(F\Delta x/kT)$ to the unfolding force dependency for each individual module results in Δx = 0.20 nm and α_0 = 1.32 x 10^{-3} s^{-1} (Domain I, pink dashed lines), Δx = 0.16 nm and α_0 = 0.97 s^{-1} (Domain III, cyan dashed lines) and Δx = 0.13 nm and α_0 = 2.1 s^{-1} (Zinc finger 2, purple dashed lines).

Figure SI4

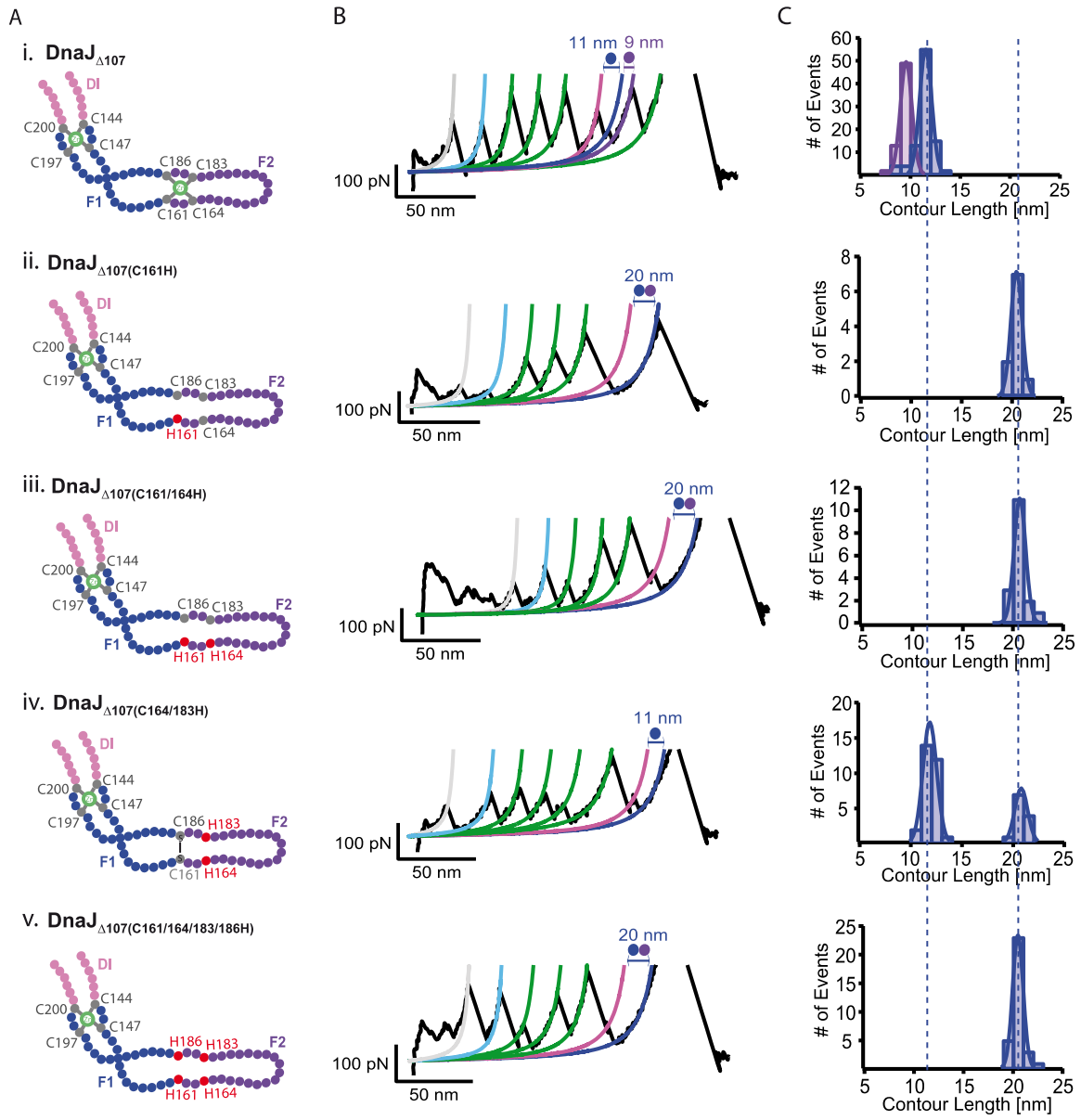

Figure SI4. The nanomechanics of the zinc finger is finely regulated by the interplay between zinc binding and disulfide bond formation. A) Schematic representation of the residues forming the second zinc finger motif in a battery of mutated forms within the $(PL)_2DnaJ_{\Delta107}(PL)_2$ context. In all cases, the first zinc finger remains unperturbed, exhibiting zinc (green sphere) binding. B) For each protein form, the resulting unfolding trajectories

reveal the mechanochemistry of the second zinc finger. C) In the wt-DnaJ$_{\Delta 107}$ (i), mechanical rupture of both individual zinc centres is fingerprinted by two consecutive events eliciting ΔL_c = 11 ± 0.6 nm (n = 92, Zf$_1$, blue) and ΔL_c = 8.9 ± 0.6 nm (n = 85, Zf$_2$, purple). By contrast, replacing one cysteine [DnaJ$_{\Delta 107(C161H)}$, (ii)] or two [DnaJ$_{\Delta 107(C161/164H)}$, (iii)] by histidines from the same chelating motif results in protein extension of ΔL_c = 19.9 ± 0.6 nm (n = 10) and ΔL_c = 19.9 ± 0.6 nm (n = 18), respectively, confirming that the second finger is devoid of mechanical stability, presumably because of the lack of Zn binding. When the two histidines are facing each other [DnaJ$_{\Delta 107(C164/183H)}$, (iv)] the presence of a disufide bond, fingerprinted by a ΔL_c = 11.4 ± 0.7 nm (n = 29), is observed in 71% of the trajectories, whereas in the remaining 29% of the occurrences the apo-form, lacking mechanical stability, was observed instead, yielding ΔL_c = 20.2 ± 0.6 nm, (n = 12). Finally, when all cysteines from Zf$_2$ are replaced with histidines [DnaJ$_{\Delta 107(C161/164/183/186H)}$, (v)], the absence of zinc binding is certified by the ΔL_c = 19.9 ± 0.5 nm (n =32) protein extension.

Figure SI5

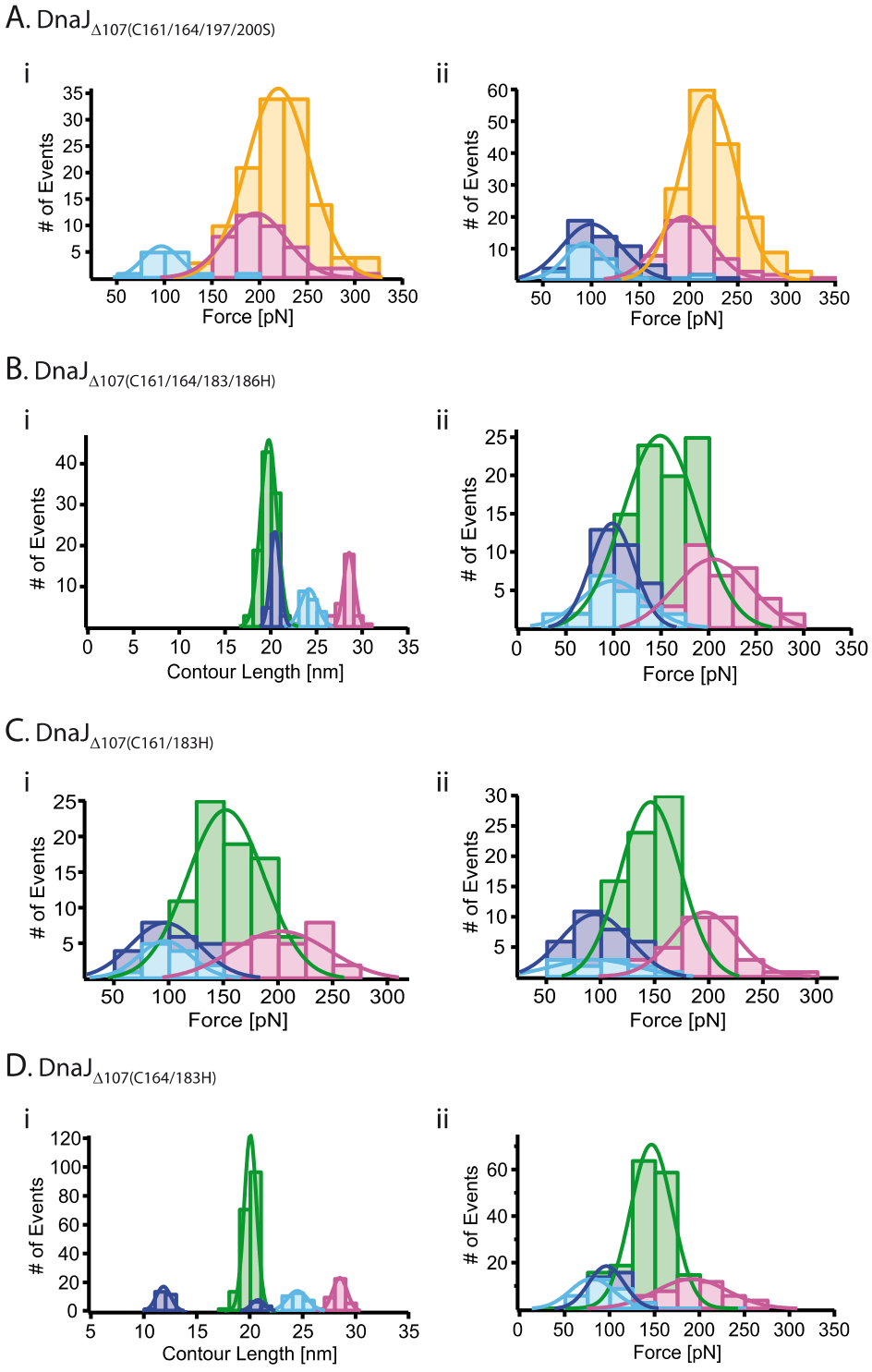

Figure SI5. Contour Length and Force histograms corresponding to the unfolding of different DnaJ$_{\Delta 107}$mutants. A) Histograms corresponding to the forces associated with the

different unfolding events observed for DnaJ$_{\Delta 107(C161/164/197/200S)}$ in its Apo (*i*) and hybrid form (*ii*). Unfolding of Domain III (cyan) for both conformations requires a force of 87.5 ± 20 pN (*n*= 13) and 81.6 ± 20 pN (*n* = 27), respectively. The unfolding of the Domain I in the Apo protein occurs at 184.7 ± 32 pN (*n* = 41), similar to the value observed for the hybrid form: 185.9 ± 28 pN (*n* = 59). The rupture of the non-native zinc finger requires a force of 92.9 ± 30 pN (*n* = 56). Finally the I27 domains unfold at ~ 208 pN ± 33 (*n* = 300). B) Histograms corresponding to the contour length increments (*i*) and forces (*ii*) observed for the unfolding of DnaJ$_{\Delta 107(C161/164/183/186H)}$. Unfolding of Domain III (cyan) requires a force of 87.1 ± 33 pN (*n* = 21) and occurs concomitant to an increase in contour length of ΔL_c = 23.5 ± 0.9 nm (*n* = 21). Domain I (pink) is characterized by ΔL_c = 27.8 ± 0.6 nm and unfolding forces of 197.1 ± 40 pN (*n* = 37). The first ZnS_4 center breaks at forces of 91.2 ± 23 pN, eliciting ΔL_c = 19.9 ± 0.5 nm (*n* =32). C) Force histograms corresponding to the unfolding of the DnaJ$_{\Delta 107(C161/183H)}$ mutant in the absence (*i*) or presence (*ii*) of 10 mM BME. The values observed for the unfolding of the different domains are similar in both conditions: 87.3 ± 23 pN (*n* = 12) and 88.9 ± 38 pN, *n* =12 (Domain III, cyan); 192.7 ± 43 pN (*n* = 29) and 183.2 ± 29 pN (*n* = 35), (Domain I, pink); 87.3 ± 31 pN (*n* = 25) and 86.2 ± 32 pN (*n* = 33), (first finger, blue) and 140.7 ± 35 pN (*n* = 83) and 133.1 ± 26 pN, *n* = 84 (PL, green). D) Histograms describing to the contour length increments (*i*) and forces (*ii*) corresponding to the unfolding of DnaJ$_{\Delta 107(C164/183H)}$. Domain III requires a force of 80.5 ± 24 pN (*n* = 34) to unfold, displaying ΔL_c = 23.8 ± 0.9 nm (cyan). The rupture of the first zinc motif (blue) occurs at 89.1± 39 pN (*n* = 41) and is fingerprinted by ΔL_c = 11.4 ± 0.7 nm (*n* = 29) when a disulfide bond between opposing cysteines is formed, and by ΔL_c = 20.2 ± 0.6 nm (*n* =12) in

the case of the apo-protein. The unfolding of the Domain I (pink) is hallmarked by a force of 181.7 ± 39 pN (n = 50) and ΔL_c = 27.8 ± 0.7 nm (n = 41). Finally the PL molecular fingerprint unfolds at 135.4 ± 23 pN (n = 182), eliciting ΔL_c = 19.5 ± 0.6 nm (green).

Table S1

DnaJ Domain	Number of Residues	Expected Length (nm)	Measured Length (nm)
Domain I	(115-143)+(201-254) 82 aa	[(82*0.36)-2.1] = 27.4	27.5 ± 0.8
Domain III	(255-325) 71 aa	[(71*0.36)-2.2] = 23.4	23.1 ± 1.0
Zinc finger 1	(144-160)+(187-200) 31 aa	[(31*0.36)-0.5] = 10.7	11.03 ± 0.6
Zinc finger 2	(161-186) 26 aa	[(26*0.36)-0.6] = 8.8	8.9 ± 0.6

Table S1. Expected and measured increment in contour length corresponding to the mechanical unfolding of each of the domains composing the DnaJ$_{\Delta 107}$ protein. For these calculations, an average value of 0.36 nm/residue was used[3].

Table S2.

	I27	ProteinL	Domain III	Domain I	Zn-Finger 1	Zn-Finger 2
$(I27)_2$ $DnaJ_{\Delta107}$ $(I27)_2$	28.3 ± 0.6 n = 143	-	23.4 ± 0.4 n = 14	-	11 ± 0.5 n = 37	9.1 ± 0.5 n = 39
$(PL)_2$ $DnaJ_{\Delta107}$ $(PL)_2$	-	19.5 ± 0.7 n = 340	23.1 ± 1.1 n = 63	27.5 ± 0.8 n = 101	11.03 ± 0.6 n = 92	8.9 ± 0.6 n = 85
$(PL)_2$ $DnaJ_{\Delta107}$ $(PL)_2$ + APPY 150 µM	-	18.9 ± 0.7 n = 314	23.1 ± 1 n = 58	27.3 ± 0.8 n = 104	11.1 ± 0.7 n = 96	8.7 ± 0.6 n = 94
$(PL)_2$ $DnaJ_{\Delta107}$ $(PL)_2$ + APPY 15 µM	-	19.6 ± 0.6 n = 472	23.5 ± 0.9 n = 77	27.7 ± 0.7 n = 138	11.1 ± 0.7 n = 136	9.0 ± 0.6 n = 136
$(PL)_2$ $DnaJ_{\Delta107}$ $(PL)_2$ + APPY 5 µM	-	19.5 ± 0.6 n = 273	23.8 ± 1 n = 38	27.6 ± 0.8 n = 95	11.1 ± 0.6 n = 95	9.2 ± 0.7 n = 91
$(Znf1Znf2\text{-}I27)_4$	27.8 ± 0.7 n = 109	-	-	-	11.2 ± 0.7 n = 82	9.1 ± 0.8 n = 72
$(PL)_2$ $DnaJ_{\Delta107(C161/164/197/200S)}$ $(PL)_2$ APO	28.1 ± 0.5 n = 124	-	23.6 ± 1 n = 13	48.1 ± 0.6 n = 41	-	-
$(PL)_2$ $DnaJ_{\Delta107(C161/164/197/200S)}$ $(PL)_2$ HYBRID	28.2 ± 0.4 n = 176	-	23.7 ± 1.2 n = 27	32.8 ± 1 n = 59	15.3 ± 0.9 n = 56	
$(PL)_2$ $DnaJ_{\Delta107(C161H)}$ $(PL)_2$	-	19.8 ± 0.6 n = 20	22.5 ± 0.6 n = 20	27.4 ± 0.7 n = 10	19.9 ± 0.6 n = 10	-
$(PL)_2$ $DnaJ_{\Delta107(C161/164H)}$	-	19.3 ± 0.9 n = 42	23.2 ± 0.3 n = 6	28.3 ± 0.6 n = 19	19.9 ± 0.6 n = 18	-
$(PL)_2$ $DnaJ_{\Delta107(C161/183H)}$ $(PL)_2$	-	19.4 ± 0.8 n = 83	23.5 ± 0.9 n = 12	27.4 ± 0.9 n = 29	11.3 ± 0.7 n = 25	-
$(PL)_2$ $DnaJ_{\Delta107(C161/183H)}$ $(PL)_2$ +BME	-	19.1 ± 0.6 n = 84	23.7 ± 1.2 n = 12	27.7 ± 0.9 n = 35	19.6 ± 0.6 n = 33	-
$(PL)_2$ $DnaJ_{\Delta107(C164/183H)}$ $(PL)_2$	-	19.5 ± 0.6 n = 182	23.8 ± 0.9 n = 34	27.8 ± 0.7 n = 41	11.4 ± 0.7/20.2 ± 0.6 n = 29 n= 12	-
$(PL)_2$ $DnaJ_{\Delta107(C161/164/183/183H)}$ $(PL)_2$	-	19.2 ± 0.9 n = 97	23.5 ± 0.9 n = 21	27.8 ± 0.6 n = 33	19.9 ± 0.5 n = 32	-

Table S2. Contour lengths averages measured for the different polyproteins used in this work. *n* indicates the number of individual events used for the analysis.

Table S3.

	I27	ProteinL	Domain III	Domain I	Zn-Finger 1	Zn-Finger 2
$(I27)_2$ $DnaJ_{\Delta107}$ $(I27)_2$	194.7 ± 36 n = 143	-	86.3 ± 22 n = 14	-	90.5 ± 31 n = 37	85.7 ± 26 n = 39
$(PL)_2$ $DnaJ_{\Delta107}$ $(PL)_2$	-	138.8 ± 24 n = 340	78.6 ± 27 n = 63	186.8 ± 39 n = 105	91.2 ± 23 n = 92	91.8 ± 20 n = 85
$(PL)_2$ $DnaJ_{\Delta107}$ $(PL)_2$ + APPY 150 μM	-	144.7 ± 32 n = 314	77.2 ± 26 n = 58	241.4 ± 35 n = 104	97.5 ± 27 n = 96	93.8 ± 23 n = 94
$(PL)_2$ $DnaJ_{\Delta107}$ $(PL)_2$ + APPY 15 μM	-	137.0 ± 32 n = 472	79.3 ± 29 n = 77	177.5 ± 26 / 247.6 ± 26 n = 142	89.0 ± 28 n = 136	85.1 ± 21 n = 136
$(PL)_2$ $DnaJ_{\Delta107}$ $(PL)_2$ + APPY 5 μM	-	133.4 ± 33 n = 273	73.1 ± 30 n = 38	176.4 ± 27 / 246.1 ± 18 n = 96	84.9 ± 27 n = 95	90.8 ± 22 n = 91
$(Znf1Znf2\text{-}I27)_4$	191.5 ± 29 n = 109	-	-	-	84.1 ± 18 n = 82	86.6 ± 16 n = 72
$(PL)_2$ $DnaJ_{\Delta107(C161/164/197/200S)}$ $(PL)_2$ APO	207.7 ± 33 n = 124	-	87.5 ± 20 n = 13	184.7 ± 32 n = 41	-	-
$(PL)_2$ $DnaJ_{\Delta107(C161/164/197/200S)}$ $(PL)_2$ HYBRID	206.8 ± 27 n = 176	-	81.6 ± 20 n = 27	185.9 ± 28 n = 59	92.9 ± 30 n = 56	
$(PL)_2$ $DnaJ_{\Delta107(C161H)}$	-	131.2 ± 15 n = 20	99.8 ± 37 n = 6	181.4 ± 10 n = 10	84.2 ± 13 n = 10	-
$(PL)_2$ $DnaJ_{\Delta107(C161/164H)}$	-	131.4 ± 21 n = 42	70.1 ± 34 n = 8	198.7 ± 39 n = 19	93.4 ± 23 n = 18	-
$(PL)_2$ $DnaJ_{\Delta107(C161/183H)}$ $(PL)_2$	-	140.7 ± 35 n = 83	87.3 ± 23 n = 12	192.7 ± 43 n = 29	87.3 ± 31 n = 25	-
$(PL)_2$ $DnaJ_{\Delta107(C161/183H)}$ $(PL)_2$ +BME	-	133.1 ± 26 n = 84	88.9 ± 38 n = 12	183.2 ± 29 n = 35	86.2 ± 32 n = 33	-
$(PL)_2$ $DnaJ_{\Delta107(C164/183H)}$ $(PL)_2$	-	135.4 ± 23 n = 182	80.5 ± 24 n = 34	181.7 ± 39 n = 50	89.1 ± 39 n = 41	-
$(PL)_2$ $DnaJ_{\Delta107(C161/164/183/186H)}$ $(PL)_2$	-	140.1 ± 38 n = 97	87.1 ± 33 n = 21	197.1 ± 40 n = 37	91.2 ± 23 n = 32	-

Table S3. Force averages measured for the different polyproteins used in this work. *n* stands for the number of individual events used for the analysis.

References

1. Schlierf, M.; Li, H.; Fernandez, J. M. The Unfolding Kinetics of Ubiquitin Captured With Single-Molecule Force-Clamp Techniques. *Proc Natl Acad Sci U S A* **2004,** *101*, 7299-7304.

2. Popa, I.; Kosuri, P.; Alegre-Cebollada, J.; Garcia-Manyes, S.; Fernandez, J. M. Force Dependency of Biochemical Reactions Measured by Single-Molecule Force-Clamp Spectroscopy. *Nature protocols* **2013,** *8*, 1261-1276.

(3) Carrion-Vazquez, M.; Oberhauser, A. F.; Fowler, S. B.; Marszalek, P. E.; Broedel, S. E.; Clarke, J.; Fernandez, J. M. Mechanical and Chemical Unfolding of a Single Protein: A Comparison. *Proc Natl Acad Sci U S A* **1999,** *96*, 3694-3699.