

# **Single Molecule Force Spectroscopy Reveals that Electrostatic Interactions Affect the Mechanical Stability of Proteins**

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## Supplementary Information

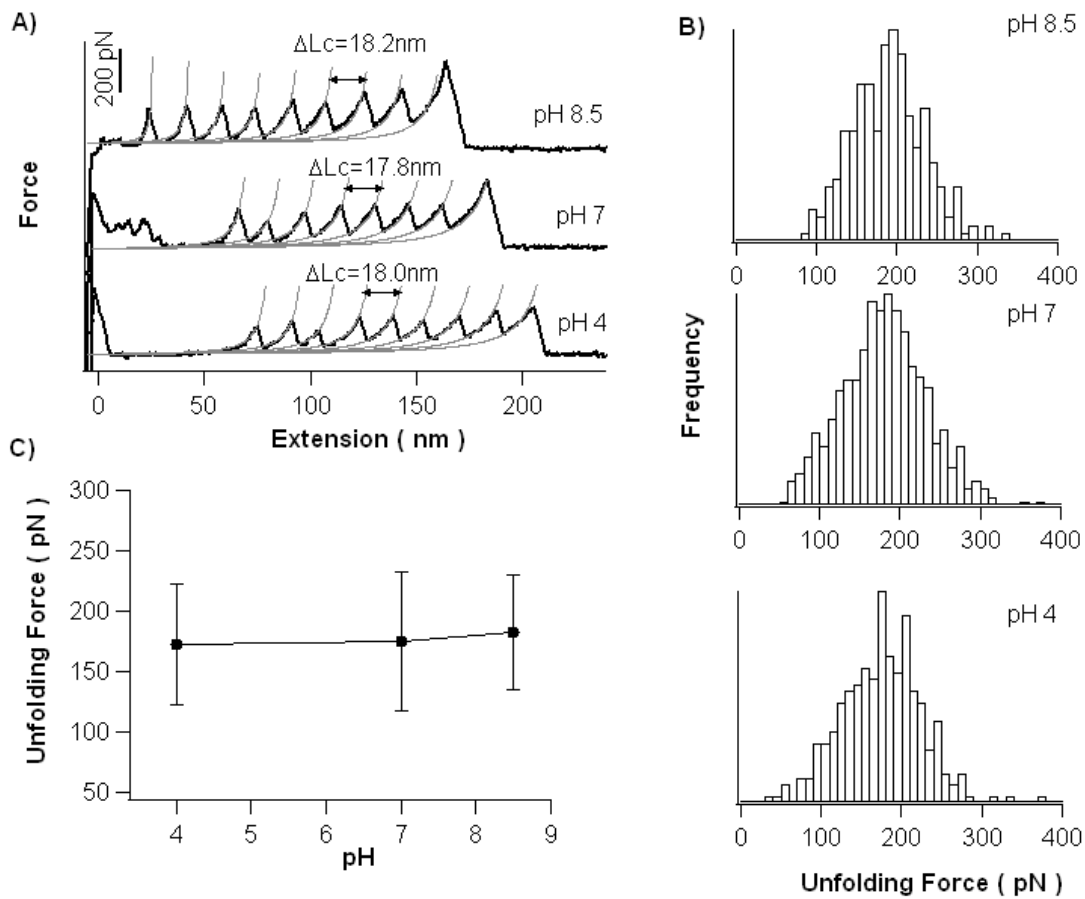


Figure 1S. Unfolding force of wild type GB1 does not show significant change in the pH range of 4-8.5. A) Typical force-extension curves of (GB1)<sub>8</sub> at different pH measured at a pulling speed of 400nm/s. Grey lines correspond to the WLC fits to the experimental data. B) Unfolding force histograms for GB1 at different pH. The average unfolding forces of GB1 are  $182 \pm 47$  pN at pH 8.5 (n=260),  $182 \pm 57$  pN at pH 7 (n=730), and  $172 \pm 50$  pN at pH 4 (n=336), respectively. C) Unfolding force of WT GB1 as a function of pH. Error bars indicate the standard deviation of the experimental data.

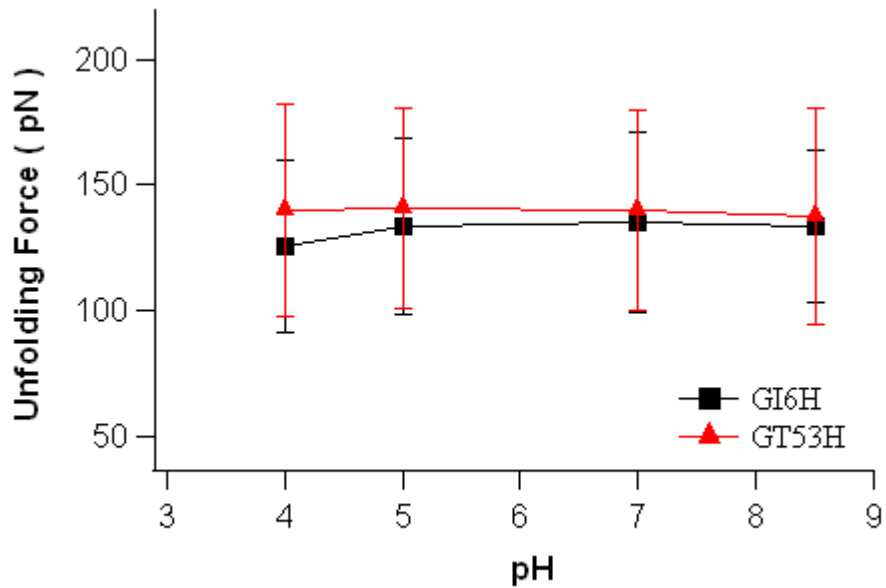


Figure 2S. Unfolding forces of single histidine mutant GI6H and GT53H do not show significant change in the pH range of 4-8.5. The average unfolding forces of GI6H are  $134 \pm 30$  pN at pH 8.5 (n=100),  $135 \pm 36$  pN at pH 7 (n=502),  $134 \pm 35$  pN at pH 5 (n=372),  $126 \pm 34$  at pH 4 (n=377), respectively. The average unfolding forces of GT53H are  $138 \pm 43$  pN at pH 8.5 (n=301),  $140 \pm 40$  pN at pH 7 (n=262),  $141 \pm 40$  (pN) at pH 5 (n=292), and at  $140 \pm 42$  pN at pH 4 (n=256). Introduction of histidine in the position 6 or 53 of GB1 does not make the mechanical stability of protein pH-dependent.