

Nucleoprotein filament formation is the structural basis for bacterial protein H-NS gene silencing

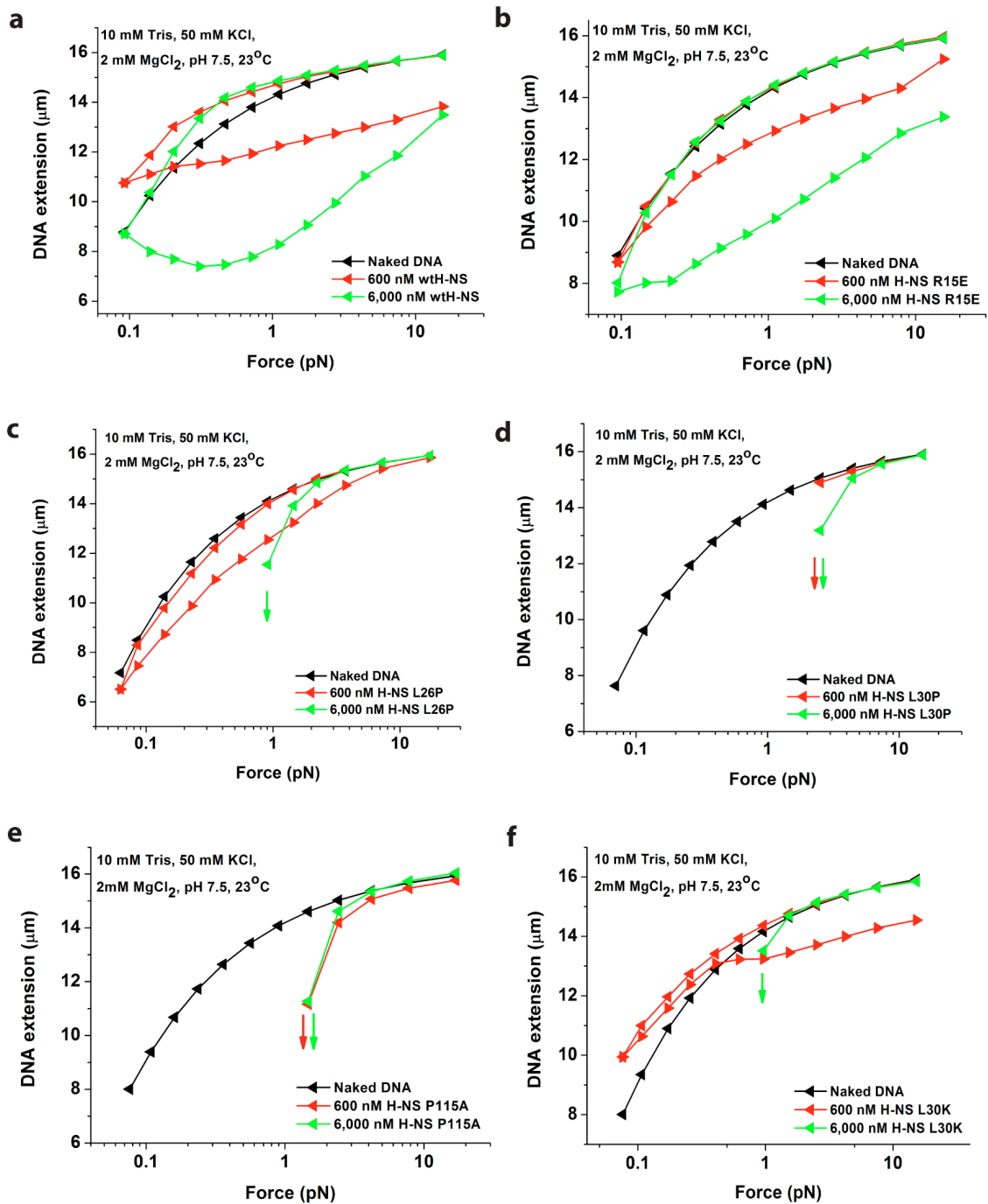
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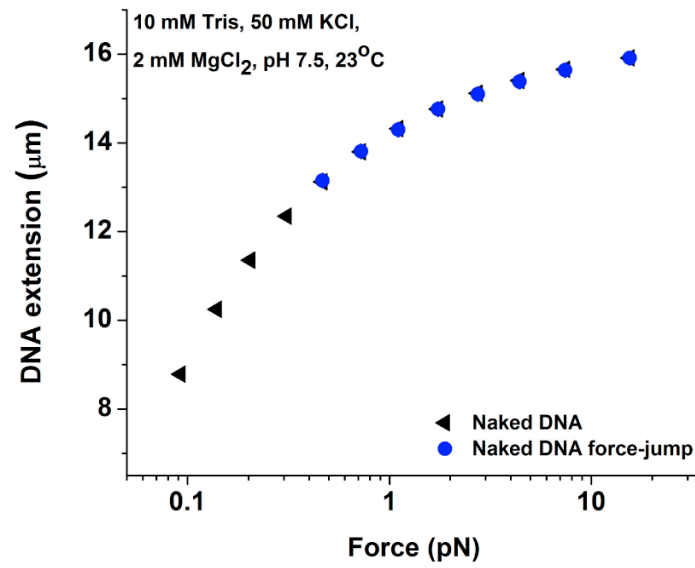
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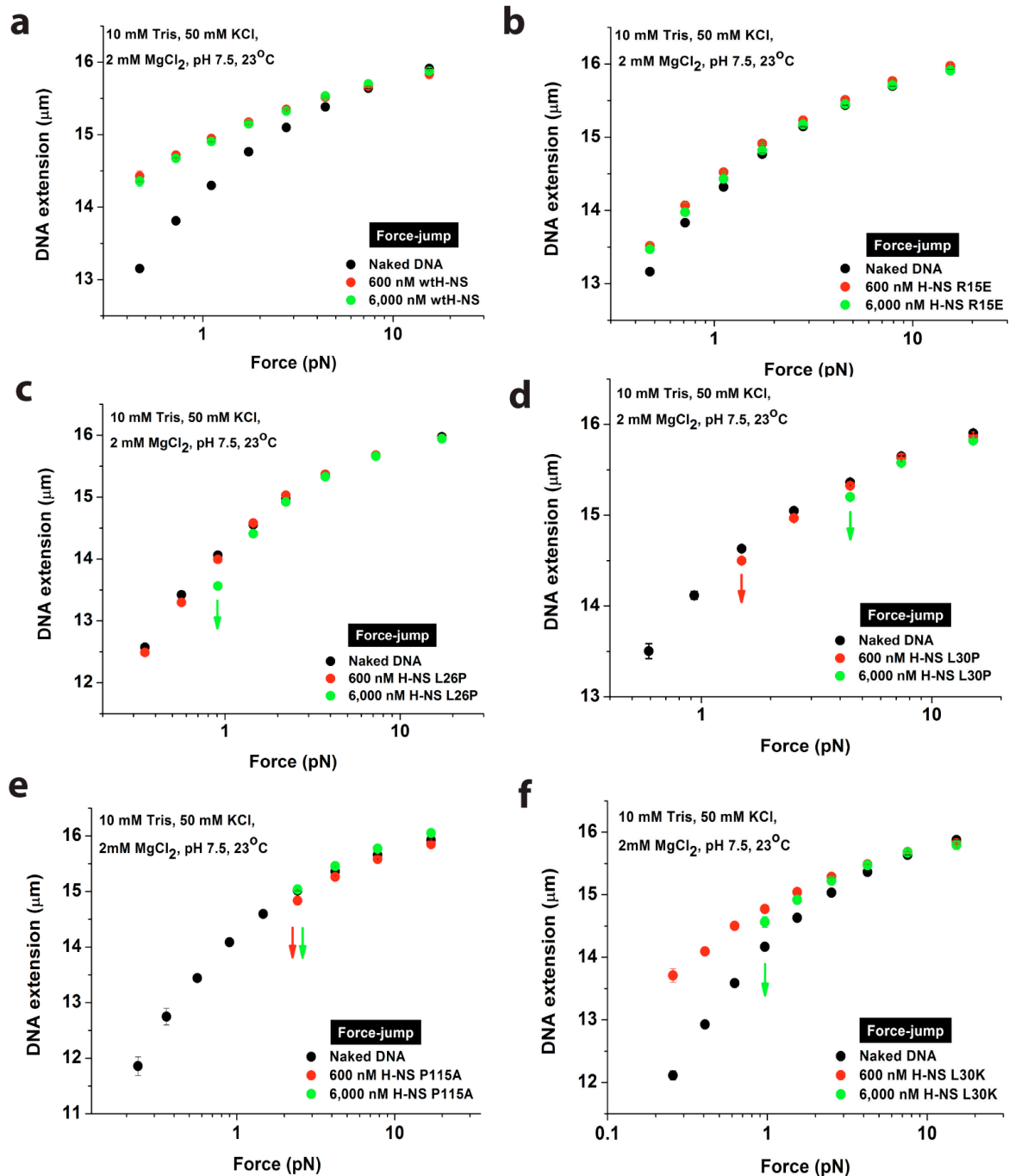
Supplementary Figures



Supplementary Figure S1 | DNA relax-stretch force-extension curves of DNA-H-NS complexes with varying protein concentration. (a) Singly-tethered λ -DNA incubated with 600 and 6,000 nM wtH-NS showed co-existence of DNA stiffening and folding, as seen from the increase in DNA extension as compared to naked DNA, and the non-overlapping between DNA relaxing (left-triangles) and stretching (right-triangles) curves respectively. (b-e) For all H-NS gene silencing negative mutants (H-NS R15E, L26P, L30P and P115A), only DNA folding were observed, either represented by DNA relax-stretch hysteresis (i.e. H-NS R15E mutant, panel b) or downwards arrow, indicating aggressive DNA folding (i.e. H-NS L30P mutant, panel d). (f) For H-NS gene silencing positive mutant, L30K, co-existence of DNA stiffening and folding was clearly observed at 600 nM protein concentration, similar to wtH-NS (panel a). At 6,000 nM H-NS L30K concentration, aggressive DNA folding was observed, indicated by downwards arrow.



Supplementary Figure S2 | Comparison of single-DNA stretching assay DNA relax-stretch approach vs. force-jump approach on naked DNA. A comparison of the DNA relax-stretch approach and force-jump approach on measuring the DNA extension of the same singly-tethered λ -DNA with respect to stretching force showed no significant change in DNA elasticity.



Supplementary Figure S3 | Force-jump force-extension curves of DNA-H-NS complexes with varying protein concentration. (a) DNA stiffening is observed for wtH-NS and the stiffening effect is saturated by 600 nM protein concentration. (b) H-NS gene silencing negative mutant, H-NS R15E, showed weak DNA stiffening, evident from the slight increase in DNA extension at the lowest probed force. DNA stiffening is also saturated by 600 nM protein concentration. (c-e) The rest of gene silencing negative H-NS mutants (H-NS L26P, L30P and P115A) showed no DNA stiffening. However, aggressive DNA folding was observed for all the 3 mutants (indicated by downwards arrows), which caused lower force measurement impossible even with the force-jump approach. (f) Gene silencing positive mutant, H-NS L30K, showed DNA stiffening and DNA stiffening was saturated at 600 nM protein concentration, as can be seen by similar DNA stiffening effect in 6,000 nM protein concentration. Aggressive DNA folding was also observed at 6,000 nM H-NS L30K, consistent with the result using DNA relax-stretch approach (Fig. S1 panel f).