Improved Single Molecule Force Spectroscopy using Micromachined Cantilevers

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Supporting Information

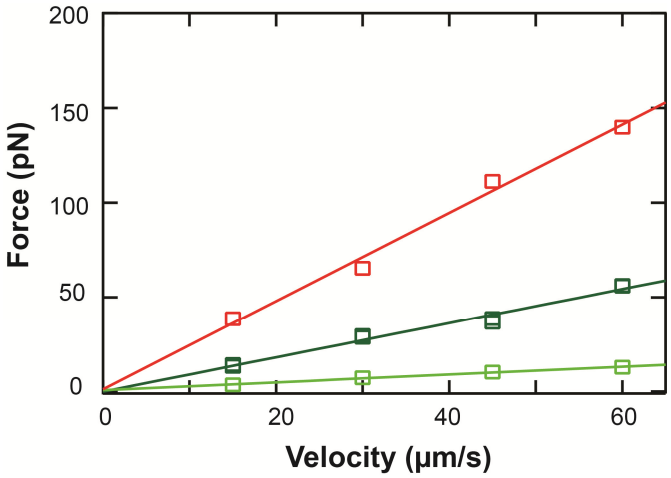

Figure S1. Measured deflection as a function of stage velocity near a surface for the three different cantilevers studies, a uncoated long BioLever (*red*), an uncoated BioLever Mini (*dark green*) and a FIB-modified BioLever (*lt green*). The cantilever was moved from in contact to the surface to 100 nm over the surface.

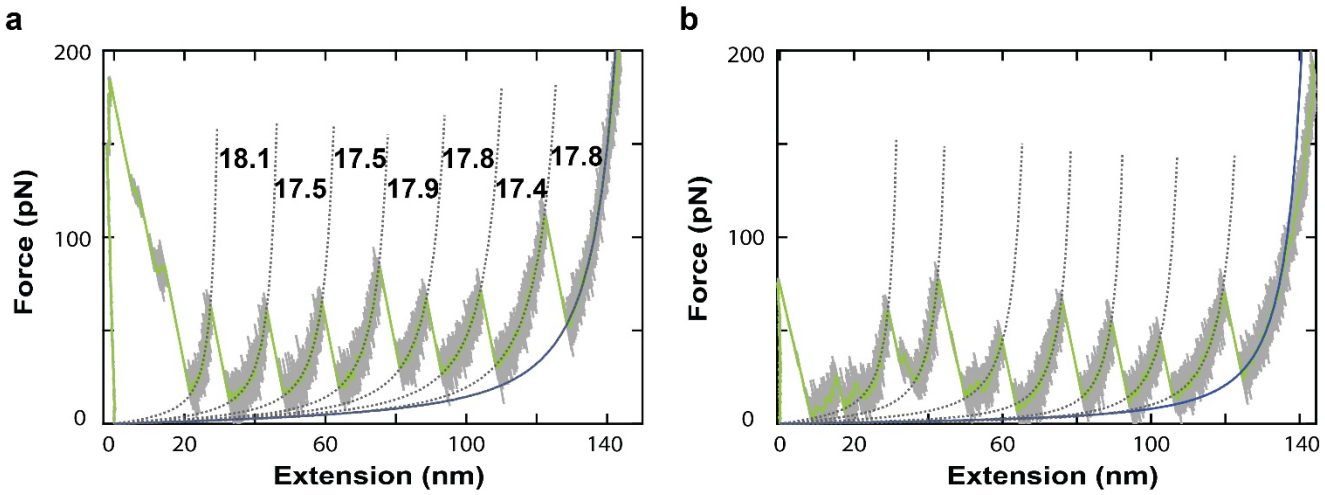

Figure S2. (a) Worm-like-chain fits to unfolding a polyprotein of NuG2. The force-extension data is shown both at high bandwidth (50 kHz, grey) and smoothed (1 kHz, green). The individual fits well describe the data. The change in contour length in nm is indicated. Our average opening distance was 17.7 nm in quantitative agreement with theoretical value of 18 nm based on as rise per amino acid (aa) of 0.36 nm/aa and the 2.1-nm distance between the ends of the polypeptide of an individual NuG2 domain.[54] (b) Force-extension curve determined using a FIB-modified cantilever on its fourth day of use in a SMFS assay, showing modified cantilevers can be reused over multiple days. Cantilevers were cleaned between applications as discussed in methods.

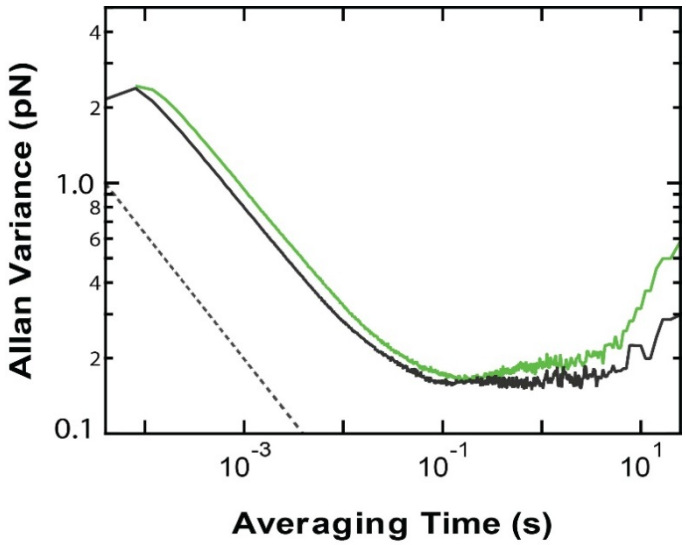

Figure S3. Comparing the Allan variance for a FIB-modified cantilever pulling on a surface-anchored protein [*green* (Figure 6b)] with that of an isolated FIB-modified cantilever [*black* (Figure 3)]. Overall, the sub-pN performance improvements were maintained during a biophysical assay and showed slight worse sub-pN performance at the longest periods. This decrease in performance most likely arises from vertical drift in the commercial AFM.