Electronic Supplementary Information

Direct Monitoring of Equilibrium Protein Folding-Unfolding by Atomic Force Microscopy: Pushing the Limit

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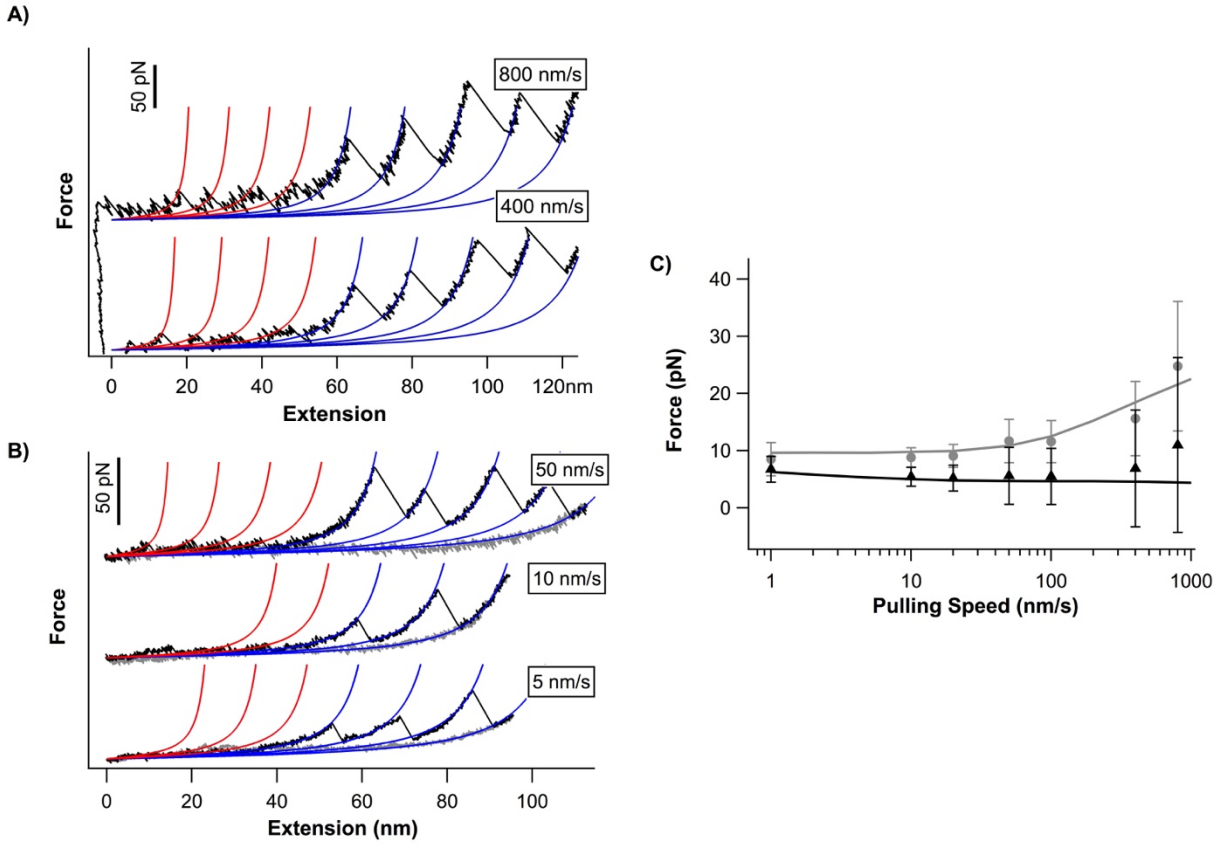

Figure S1. The mechanical unfolding and refolding of $(NuG2\text{-}GA)_4$ under constant velocities. A) Typical force-extension curves at pulling speeds of 400 nm/s and 800nm/s. The hopping between the folded and unfolded states of GA is clearly visible. WLC fittings (solid lines) are shown for GA (red) and NuG2 (blue). B) Representative stretching (black) and relaxation (gray) curves at various pulling velocities. WLC fittings (solid lines) are shown for GA (red) and NuG2 (blue). C) Pulling speed dependency of folding (black) and unfolding (gray) forces. Solid lines show Monte Carlo simulation results initialized by the following parameters: Δx of 1.1 nm and 4.5 nm; k_0 = $0.31s^{-1}$ and $1*10^5$ s^{-1} for unfolding and folding respectively.

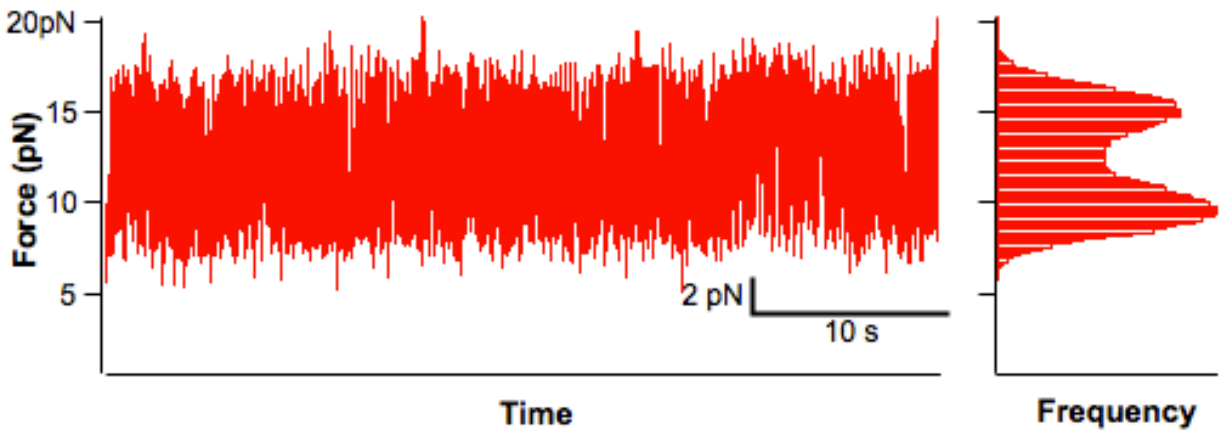

Figure S2. Constant separation experiment of GA demonstrating stability at a fixed stage position over ~40 seconds. Right: population distribution of folded (higher force) and unfolded (lower force) states.

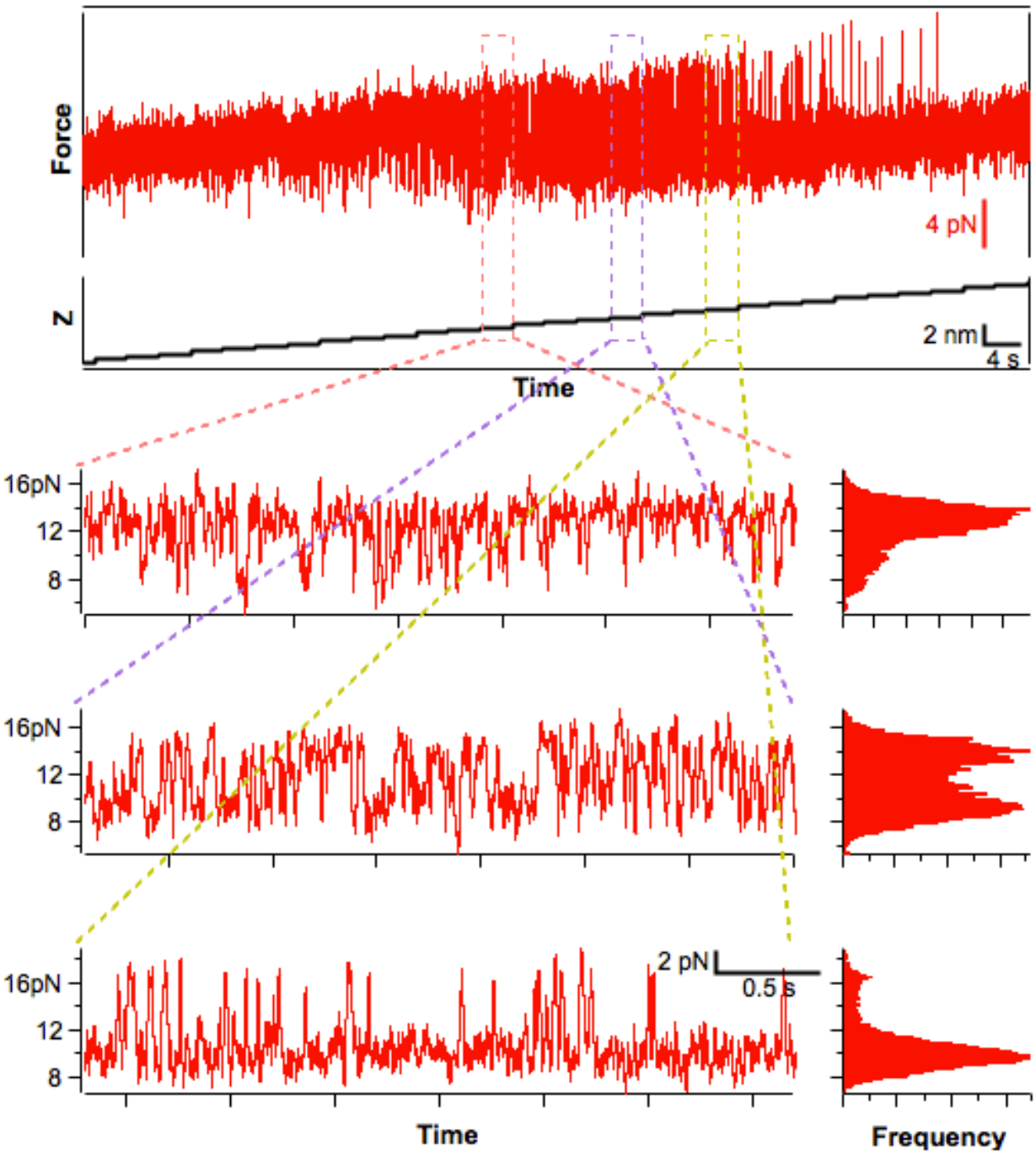

Figure S3. Constant separation trace from Fig 2. Insets show zoomed in regions of different fixed stage positions demonstrating the effect of stretching on GA folding/unfolding equilibrium. At low extensions, GA favours the folded state (top). As the extension increases, the unfolded state becomes increasingly populated (middle, bottom).

Materials and Methods

Protein Engineering

The gene encoding GA is a kind gift from Prof. Bryan. The polyprotein gene (NuG2-GA)4 was constructed according well-established, previously reported protocols.[1, 2] The gene was cloned into pQE80L expression vector and transformed into DH5α competent E. coli. Overexpression of target protein was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG; Invitrogen). The target protein was purified by Co2+ affinity chromatography. The purified protein was stored in pH 7.4 phosphate-buffered saline (PBS) at 4 °C ready for SMFS experiments.

Single Molecule Force Spectroscopy

Chemical etching of cantilevers was conducted to remove gold and underlying chromium coatings by previously described methods.[3] Briefly, Gold and underlying chromium were chemically etched from cantilevers using commercial etchants (Sigma-Aldrich). The cantilevers were first submerged in gold etchant for 30 s and then rinsed with deionized water. The cantilevers were then submerged in chromium etchant for 30 s and rinsed again with deionized water.

SMFS experiments were conducted on a commercial AFM (Cypher, Asylum Research). Before each experiment, the spring constant (~5 pN/nm) of the silicon nitride cantilevers (BioLever, Olympus JP) were calibrated using the thermal method in PBS solution.[4] Typically, 1-3 μL of protein (~1.0 mg/mL) was added to a clean glass coverslip covered in ~100 μL PBS and allowed to adsorb for ~20 min. In a typical constant velocity SMFS experiment, the cantilever was brought into 'hard' contact (~300 pN) with the surface to induce molecule attachment, then retracted at a constant velocity (e.g. 400 nm/s). The curves were then screened for single molecule stretching (~1% of all curves). Only these curves were analyzed.

Constant separation experiments were conducted using procedures adapted from an open-source procedure[5, 6] Molecules were first picked up manually by manipulating the sample stage as in a typical fishing experiment. Only after a single molecule was confirmed through high speed stretching were equilibrium measurements attempted. Follow-up stretching confirmed that the same single molecule remained. Data analyses and Monte Carlo simulations were carried out using in-house software written for Igor Pro (WaveMetrics, Inc.).

Monte Carlo simulations were performed to reproduce our experimental data to determine unfolding kinetics.[7] The force of stretching the polypeptide was calculated by the worm-like chain (WLC) interpolation formula:[8] $F(\mathrm{x}) = (k_\mathrm{B}T/b)\,[0.25(1 - x/L)^{-2} - 0.25 + x/L]$, where k_B is the Boltzmann constant, T is the temperature, b is the persistence length and L is the contour length. The Bell-Evans model was used to calculate the probability distribution of unfolding and folding:[9, 10] $k(F) = k_0 * \exp(\pm F\Delta x^{\ddagger}/k_\mathrm{B}\mathrm{T})$, where T is the temperature and the positive and negative exponent apply for unfolding and folding rates

respectively. The quality of the best fit was determined by a χ^2 analysis within a small parameter space. Therefore, the error associate with such fits is relatively large,[11] for Δx, the error is around 10%, and for rate constant, it is typically within a factor of 3.

References

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