

Sequence-dependent elasticity and electrostatics of single-stranded DNA: Signatures of base stacking

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1 Force data smoothing

Most of the noise in the force-extension data comes from the measurement of the force. While the extension measurement depends on the average of measurements of the extension over time $\langle X(t) \rangle$, the force requires estimate of the variance in fluctuations of the lateral signal position $\langle y(t)^2 \rangle$ which has a significantly larger error (1).

For many applications, particularly data visualization, it is useful to smooth the force measurements in the force-extension curve (see Supp. Fig. 1). In order to do this we fit a power law to the logarithm of the force as a function of the magnet position in the magnetic tweezers assay:

$$\log f = \sum_{i=0}^4 b_i z_{mag}^i, \quad (1)$$

where z_{mag} is the magnet position and b_i are the fit parameters (see Supp. Fig. 1A). Replacing the measured forces with this function evaluated at the measured magnet position effectively smooths the forces locally. The results are generally insensitive to the order of the polynomial used: varying the order from 3 – 6 has little effect, but lower orders lead to systematic deviations from the data while higher orders begin to interpolate the noise. Smoothing near the endpoints is avoided by using a clipping function:

$$f_{replace}(z_{mag}) = G(z_{mag})f_{fit}(z_{mag}) + (1 - G(z_{mag}))f_{measured}(z_{mag}), \quad (2)$$

where $f_{measured}$ is the measured force, f_{fit} is the polynomial fit evaluated at z_{mag} , and $G(z_{mag})$ is a function that is 1 throughout the central range of z_{mag} and varies smoothly between 0 and 1 near the endpoint (see inset of Supp. Fig. 1B) – here we have used a sinusoidal function that transition from 0 to 1 over 1 mm in z_{mag} .

2 Data reproducibility and scaling of extension data

As a result of the polydispersity of contour length associated with the rolling circle amplification reaction as well as the nonspecific tethering strategy, different tethers have varying contour lengths. When scaled by the extension at 55 pN, force-extension behavior is quite reproducible from tether to tether (see Fig. 2A) indicating that the elastic behavior is extensive in contour length and there are no finite-length effects.

Full force-extension behavior over approximately 3 orders of magnitude is measured using a two different beads: myone and M280 (Dynabeads). In order

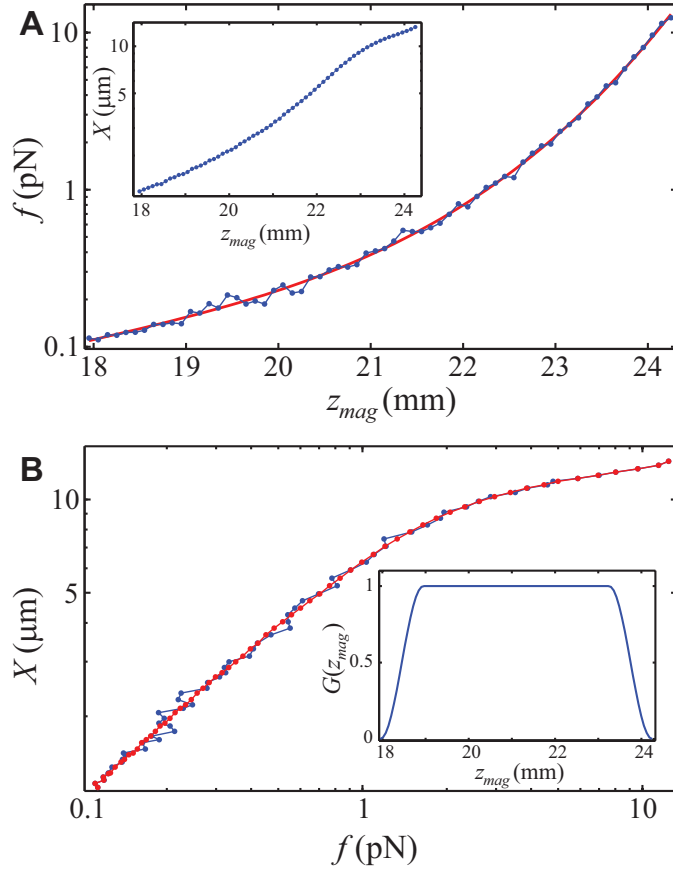


Figure 1: Smoothing of force significantly improves data visualization. A. Measured forces as a function of magnet position with fourth order polynomial fit (Eqn. 1). The inset is a plot of the average extension as a function of magnet position, which is has considerably less noise. B. The force-extension curve before (blue) and after (red) the smoothing protocol described in the text. The inset is the smoothing function that avoids smoothing at the end points – see text for details.

to connect the low-force data with the high-force data we scale the low-force extensions so as to maximize the overlap with the high-force extensions (see Fig. 2B). To do this we find the region of overlap (see shaded region in Fig.2B), interpolating the high- and low-force extensions at 50 logarithmically spaced points in force, the ratio of the two interpolated extensions gives the scaling factor. We collapse the curves by multiplying the low-force extension data by the average of these 50

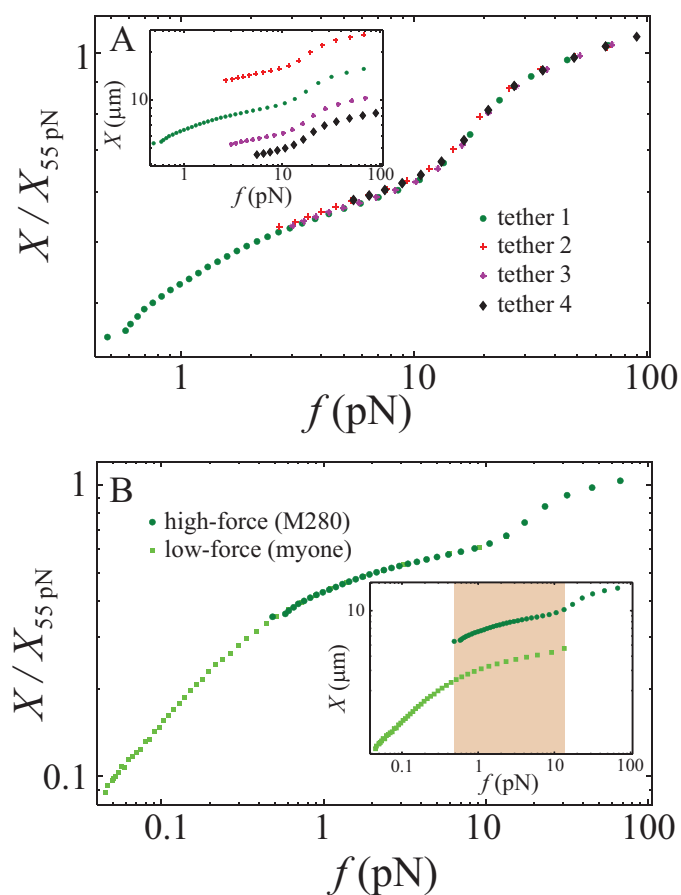


Figure 2: Data scaling. A. Four different tethers from four separate experiments in 10 mM tris 20 mM NaCl at high force (with M280 beads) all reliably scale onto one another when normalized at 55 pN. Inset: The unscaled force-extension data shows the variability in contour length of the molecules. B. Rescaling of low-force data to maximize overlap between two different molecules - one at low force and one at high force. Overlap of the data is quite good over a broad range of forces. Inset: Before scaling the low-force data, a discrepancy in contour length is apparent. The region of force overlap is shaded.

scaling factors.

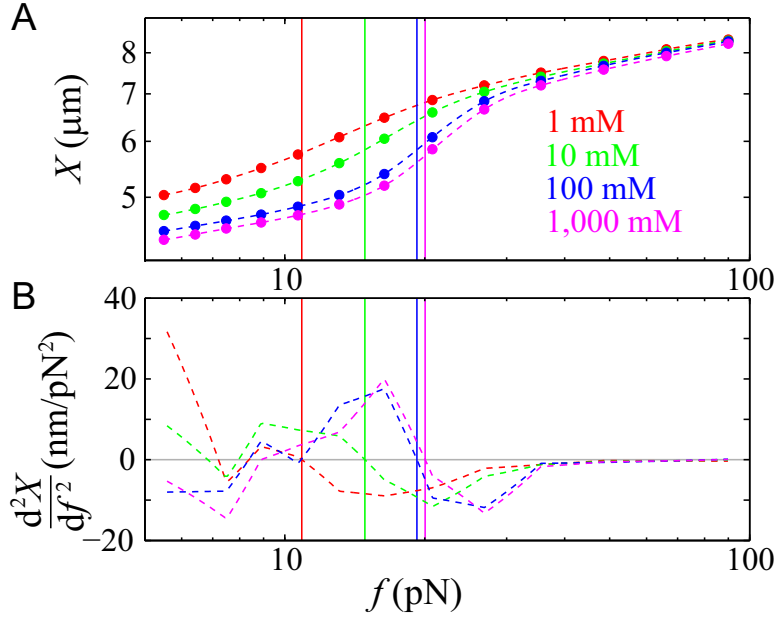


Figure 3: Procedure for extracting the force at the unstacking transition in poly(dA). A. Data at each salt concentration are interpolated with a spline function (dashed curves). The largest force for which this quantity vanishes is the force at the unstacking transition (marked here with vertical lines). B. The second derivative of the spline interpolating functions from A.

3 Extracting the electrostatic tension

In order to extract the applied force at the midpoint of the helix-to-coil transition in the poly(dA) data, we estimate the point at which the curvature of the data vanishes. To do this we begin by isolating the high force M280 data at each salt concentration. This data is smoothed as described above in Section 1 and then fit to an interpolating spline function (see Fig. 3A). The spline function is the output of the default `spline` function in matlab. It fits a cubic function between every two consecutive points along the curve, forcing the function and its first derivative to be continuous and smooth at each measured (f, X) point. Thus, the second derivative of the spline function is continuous – a crucial element of our approach. We evaluate the second derivative of X with respect to f – a piecewise linear function (see Fig. 3B). The largest force for which this function changes sign from positive to negative defines our unstacking transition ($f_{applied}$).

References

1. Ribeck, N., and O. A. Saleh, 2008. Multiplexed single-molecule measurements with magnetic tweezers. *Rev. Sci. Instrum.* 79:094301–6.