Supplementary Material

Experimental Setup. For data acquisition and analysis we used a dt 3016 board (DataTranslation, MA, USA) and a modified software version from Asylum Research, Santa Barbara, CA. Calibration of the spring constant was done in solution using the equipartition theorem. For all experiments we used biolevers (Olympus Optical CO., Tokyo, Japan) with spring constants between 5 and 8 pN nm^{-1}. A drop of protein solution (PBS buffer at a protein concentration of 0.1- 3 mg/ml) was placed on a freshly evaporated gold surface and incubated for 5 min. Subsequently the sample was thoroughly rinsed with PBS buffer to remove excess protein. All measurements were performed in PBS buffer (135 mM NaCl, pH 7.4).

Determination of unfolding forces. To minimize errors in the unfolding force histograms shown in Figure 4 we used the following protocol to measure unfolding forces: in a first step we fitted the interpolation formula for the worm like chain model of entropy elasticity given by Bustamante et al. to our data (Bustamante et al., 1994). Persistence length used was in a range between 0.7 to 1 nm. In a second step we fitted a line to the relaxation phase of the cantilever immediately following the unfolding event. The intersection point between the two fits was taken as the unfolding force F. Due to the high pulling velocities of v= 2 μm/s it is important to correct the measured unfolding forces ($F_{measured}$) for hydrodynamic forces acting directly on the cantilever spring. The actual pulling velocity prior to the unfolding event depends on the ratio of the spring constant of the cantilever spring k_{lever} and the spring constant of the polymer spacer s. Since the polymer spacer spring is non-linear the actual spring constant s was deduced from the slope of the WLC-fit immediately before the unfolding event. We obtained corrected force values F_{real} from the equation $F_{real}=F_{measured}+F_{fric}*(s/(k_{lever}*s)$. F_{fric} is the half the value of the force hysteresis between an approach curve and a retraction curve of a free untethered cantilever.

References

Bustamante, C., Marko, J.F., Siggia, E.D. and Smith, S. (1994) Entropic elasticity of lambda-phage DNA. *Science*, **265**, 1599-1600.