Single Molecule Localization and Discrimination of DNA–Protein Complexes by Controlled Translocation Through Nanocapillaries

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Through the use of optical tweezers we performed controlled translocations of DNA–protein complexes through nanocapillaries. We used RNA polymerase (RNAP) with two binding sites on a 7.2 kbp DNA fragment and a dCas9 protein tailored to have five binding sites on λ-DNA (48.5 kbp). Measured localization of binding sites showed a shift from the expected positions on the DNA that we explained using both analytical fitting and a stochastic model. From the measured force versus stage curves we extracted the nonequilibrium work done during the translocation of a DNA–protein complex and used it to obtain an estimate of the effective charge of the complex. In combination with conductivity measurements, we provided a proof of concept for discrimination between different DNA–protein complexes simultaneous to the localization of their binding sites.