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Thermodynamic DNA Looping by a Two-Site Restriction Endonuclease Studied using Optical Tweezers GREGORY J. GEMMEN, RACHEL MILLIN, DOUGLAS E. SMITH, UCSD — Many enzyme-DNA interactions involve multimeric protein complexes that bind at two distant sites such that the DNA is looped. An example is the type IIe restriction enzyme Sau3AI,which requires two recognition sites to cleave the DNA. Here we study this process at the single DNA level using force measuring optical tweezers. We characterize cleavage rates of single DNA molecules in the presence of Sau3AI as a function of enzyme concentration, incubation time, and the fractional extension of the DNA molecule. Activity is completely inhibited by tensions of a few picoNewtons. By replacing Mg^{2+} with Ca^{2+}, the Sau3AI dimers form but do not cleave the DNA, thus trapping DNA loops. We are able to pull apart these loops, measuring the force needed and the length of DNA released for each. We also characterize the number and length distributions of these loops as a function of incubation time and DNA fractional extension. The results of these studies are discussed in the context of a Brownian dynamics model of DNA looping.

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