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A survey of DNA looping and cleavage properties of different restriction enzymes using optical tweezers RACHEL MILLIN, GREGORY J. GEMMEN, DOUGLAS E. SMITH, UCSD — Of the more than 3500 known Type II REases, a small but growing number have been identified that require two copies of the enzyme's recognition site for activity. Each site is bound to one enzyme subunit, and the two subunits come together by thermodynamic DNA looping to form an active multimer that cleaves the DNA. When Ca^{++} is replaced with Mg^{++}however, the multimers usually "staple" the recognition sites together trapping the DNA loops. Using force measuring optical tweezers, we investigate the behavior of 16 different two-site REases from the Type IIe, Type IIf, and Type IIs subsets on single DNA molecules in the presence of Mg^{++}, Ca^{++}, and EDTA. We show that one-site and two-site REases may be rapidly discerned. By measuring the force needed to disrupt the loops in the presence of Ca^{++}, we elucidate various binding behaviors amongst the two-site REases, probing DNA-enzyme and/or enzymatic subunit-subunit affinity. For one enzyme, HpaII, the effect of $[Ca^{++}]$ on activity is studied in detail.

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