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Measuring Homologous Pairing Using Synthetic Dna Constructs

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In homologous recombination, DNA segments are exchanged between chromosomes, a process critical to establishing genetic variation and healing DNA damage. To avoid detrimental effects, cells must ensure that homologous, not heterologous, segments are exchanged. Prior to exchange, however, pairing must occur; in the chaos of the cell nucleus, how do homologous segments find each other? Here, by measuring dsDNA pairing in synthetic constructs, we test the hypothesis that homologous pairing can be electrostatically-driven in a protein-free environment. Since the electrostatic-pairing theory predicts homologous recognition to be weak, we tether duplexes together to entropically-bias their pairwise interactions, creating so-called DNA 'scissors'. The physiologically-relevant, divalent cations of magnesium and calcium are known to specifically adsorb on anionic dsDNA, the resulting charge compensation reduces dsDNA-dsDNA electrostatic repulsion. Accordingly, for our DNA scissors, coaligned duplexes are observed for sufficient concentrations of these cations, an effect that disappears when duplex tethering is absent. By varying the nucleotide sequences of the duplexes-of-interest, the magnitude of divalent-cation-induced interactions between homologous and heterologous duplexes can be compared and the effect of electrostatically-driven homologous recognition can be quantified.