High affinity and un-dissociated capillary electrophoresis for measuring protein-DNA interactions and for DNA separation

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DNA strange exchange reaction involving ssDNA and homologous dsDNA is the central step of homologous recombination, which can be utilized biologically for gene editing and chemically for recombinase-polymerase isothermal amplification and signal amplification in point-of-care diagnostics. In this study, we describe the development of a unique, super-stable protein–DNA-affinity system using a high electric field and demonstrated, for the first time, free-solution capillary electrophoresis for rapid and sensitive detection of fundamentally important DNA strand exchange reactions, including detection of single DNA mismatches caused by replication errors. Furthermore, application of this assay allowed identification of two engineered hyper-recombinases useful for enhancing strand-exchange efficiency. These innovations offer promising and diverse applications for the development of new separation-reaction-coupled chemical tools.