A new multiplexing single molecule technique for measuring restriction enzyme activity

ALLISON HARBOTTLE, JILLIAN CAVANAUGH, Emmanuel College, Boston, MA, WENDY GORDON, JOSEPH LOPARO, Harvard Medical School, Boston, MA, ALLEN PRICE, Emmanuel College, Boston, MA — We present a new multiplexing single molecule method for observing the cleavage of DNAs by restriction enzymes. DNAs are attached to a surface at one end using a biotin-streptavidin link and to a micro bead at the other end via a digoxigenin-antidigoxigenin link. The DNAs are stretched by applying a flow. After introduction of the restriction enzyme, the exact time of cleavage of individual DNAs is recorded with video microscopy. We can image hundreds to thousands of DNAs in a single experiment. We are using our technique to search for the signature of facilitated diffusion in the measured rate dependence on ionic strength.