

14e Single Molecule Kinetics of Reverse Transcriptase

Charles M. Schroeder, Sangjin Kim, Paul C. Blainey, and X. Sunney Xie

We use single molecule methods to directly observe the enzymatic behavior of individual reverse transcriptase (RT) molecules on single-stranded DNA template strands *in vitro*. A flow-stretched DNA assay is used, allowing for characterization of enzymatic rate, processivity, and pausing of RT during DNA synthesis as a function of template base pair content, secondary structure, and applied template tension. We study the kinetics of plus-strand DNA synthesis catalyzed by RT derived from the Moloney murine leukemia virus (M-MLV) with inhibited RNase H activity, an enzyme commonly used for RT-PCR. Initial observations show an average enzymatic rate of ~5-10 bp/sec. Understanding potentially diverse kinetic mechanisms in molecular subpopulations may allow for development of more effective treatments for retroviral infections leading to cancer.