

POSTER 16**SLIDE INTO ACTION: DYNAMIC SHUTTLLING OF HIV REVERSE TRANSCRIPTASE ON NUCLEIC ACID SUBSTRATES**

Shixin Liu¹, Elio A. Abbondanzieri¹, Jason W. Rausch⁴, Stuart F. J. Le Grice⁴, and Xiaowei Zhuang^{1,2,3}

¹Department of Chemistry and Chemical Biology, ²Department of Physics, ³Howard Hughes Medical Institute, Harvard University, Cambridge, MA 02138, USA; ⁴HIV Drug Resistance Program, National Cancer Institute, Frederick, MD 21702, USA

Reverse transcriptase (RT) of human immunodeficiency virus (HIV) catalyzes a complex series of reactions to convert single-stranded viral RNA into double-stranded DNA for host cell integration. A variety of enzymatic activities, including DNA synthesis, RNA cleavage, strand transfer, and strand displacement synthesis, are performed by RT to complete conversion of the viral genome. Crystal structures, biochemical assays and single-molecule analyses have suggested different modes of interaction between RT and nucleic acid duplexes, providing incisive snapshots of the nucleoprotein complexes that illuminate the functional mechanism of RT. Nevertheless, how the enzyme acquires specific functional configurations on nucleic acid substrates and how it switches between different functional modes remain unclear. For example, how does RT efficiently locate the 3' terminus of the nascent DNA primer on a long duplex substrate to initiate DNA polymerization? This question is particularly important for a low-processivity polymerase such as RT, which must frequently re-locate the polymerization site following dissociation in order to complete conversion of the entire HIV genome. More puzzling is how the dissociated RT re-locates the polymerization site during strand displacement synthesis, considering that the primer terminus may itself be displaced from the template by the competing non-template strand. Also, RT has been shown to cleave at many different sites within a DNA/RNA hybrid, but how RT accesses these sites remains incompletely understood. A dynamic visualization of how RT interacts with different substrates in real time will help us to address these questions and gain a more complete understanding of its function.

In this work, we extended our fluorescence resonance energy transfer approach [1] to monitor the action of individual HIV-1 RT molecules and their interactions with nucleic acid substrates in real time. RT was observed to slide over long distances on nucleic acid duplexes, allowing the enzyme to rapidly shuttle between opposite termini of the duplexes and sample different sites on substrate. Sliding dynamics were regulated by cognate nucleotides and anti-HIV drugs targeting RT. These long-range translocation activities facilitate multiple stages of the reverse transcription pathway, including both normal DNA polymerization and strand displacement synthesis [2].

[1] Abbondanzieri *et al.*, (2008) *Nature* 453, 184

[2] Liu *et al.*, (2008) *Science*, *in press*