

## SINGLE MOLECULE STUDIES REVEAL SLIDING DYNAMICS OF HCV NS5B IN COMPLEX WITH RNA

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NS5B is an RNA-dependent RNA polymerase capable of initiating RNA synthesis *de novo*. However, the detailed underlying mechanism remains elusive. In particular, it is unclear how the enzyme locates the 3'-terminus of the RNA template. Previous studies suggested that the nucleic acid binding channel of NS5B accommodates approximately 10 residues of a single stranded RNA. Although the contacts between the polymerase and its nucleic acid substrate are maximized, the 3'-end of the primer is not properly positioned under these conditions and such complexes are therefore unproductive. Hence, it is conceivable that the NS5B-RNA interaction is highly dynamic. A given population of complexes may exist as a mixture of a productive and various distinct unproductive species. Of note, nonnucleoside inhibitors of NS5B were shown to inhibit formation of a competent complex. To address this problem, we have conducted single molecule FRET (SM-FRET) experiments. This approach allowed us to obtain a direct visualization of both the positioning and dynamics of NS5B in complex with its RNA template. We performed our experiments on single-donor (Cy3)/acceptor (Cy5) fluorophore-labeled RNA substrates, which were surface-immobilized to enable long observation times. Binding of NS5B caused a significant increase in FRET. Experiments conducted with mutant forms of NS5B, modified at the RNA entry site, did not show changes in FRET. Most importantly, SM-FRET studies on RNA-protein complexes revealed protein sliding dynamics occurring in the millisecond time scale. These dynamics change with the RNA template length, and with the presence of complementary DNA strands that restrict the motion of NS5B. A nonnucleoside inhibitor is observed to compromise either binding of NS5B and/or sliding of the polymerase along the template. Taken together, our single molecule studies provide for the first time direct evidence for the ability of NS5B to slide along its RNA template. Sliding of NS5B provides a plausible mechanism that facilitates formation of a productive complex. Conversely, interference with these dynamics provides a possible mechanism by which nonnucleoside analogue inhibitors of NS5B block *de novo* initiation of RNA synthesis.