reflection and confocal excitation. By using a FRET pair with dyes at the ends of the DNA substrates we are able to follow the bending dynamics of the substrates at the single molecule level. We carried on experiments with duplex DNA 15 bp and 18 bp long to determine binding affinity and potential binding of multiple units to the substrate. DNA substrates containing bulges were used for comparison to study the effect of pre-bent structures on binding affinity and conformational dynamics. Bending of the DNA substrates is observed by changes in FRET efficiency allowing determining the conformational dynamics of the system in real time with temporal resolution in the order of milliseconds.

1885-Plat
Slide into Action: Dynamic Shuttling of HIV Reverse Transcriptase on Nucleic Acid Substrates
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The reverse transcriptase (RT) of human immunodeficiency virus (HIV) catalyzes a series of reactions to convert single-stranded viral RNA into double-stranded DNA for host cell integration. This process requires a variety of enzymatic activities, including DNA polymerization, RNA cleavage, and strand displacement synthesis. As a major target for anti-HIV therapy, RT has been the subject of extensive research. Nonetheless, how the enzyme-substrate complex acquires specific functional configurations and switches between different functional modes remains unclear. Here, we used single-molecule fluorescence resonance energy transfer to probe the interactions between RT and nucleic acid substrates in real time (1). Surprisingly, RT was observed to slide on nucleic acid duplexes, rapidly shuttling between opposite termini of the duplex. Sliding kinetics were regulated by cognate nucleotides and non-nucleoside RT inhibitors, a major class of anti-HIV drugs, which stabilized and destabilized the polymerization mode, respectively. These long-range translocation activities facilitate multiple stages of the reverse transcription pathway. First, sliding allows RT to target the polymerization site by one-dimensional search. Remarkably, upon reaching the polymerization site, an RT molecule originally bound in the opposite orientation can spontaneously flip into the polymerization orientation without dissociation, enhancing the target search efficiency. Furthermore, sliding helps RT to actively disrupt secondary structures on the substrate and kinetically access the polymerization site, thereby facilitating strand displacement synthesis. It is remarkable that an enzyme could have such large-scale translational and orientational dynamics. This type of dynamic flexibility may be a general design principle for multi-functional enzymes, helping them to rapidly access different configurations required for different functions.
Reference:
1. S. Liu, E.A. Abbondanzieri, J.W. Rausch, S.F.J. Le Grice, X. Zhuang, ''Slide into action: dynamic shuttling of HIV reverse transcriptase on nucleic acid substrates'', Science (in press).

1886-Plat
Single Molecule Study Of the RNA Degradation and Polyadenylation Activities of the Archaeal Exosome
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RNA biosynthesis involves a certain frequency of errors due to inherent inaccuracies of enzymes involved. Although DNA damage is repaired, RNA errors are rapidly eliminated. In particular, the degradation of 3' poly A tail is very important in RNA metabolism to maintain the fidelity of mRNA synthesis in the cell. A key enzyme involved in both RNA processing and RNA degradation is the exosome complex. The archaeal exosome can function in reverse reactions as either an RNA polymerase or an RNA exoribonuclease. In the presence of free inorganic phosphate (P_i), the exosome phosphorolytically degrades RNA substrates from the 3' end, whereas in the presence of ADP, it polymerizes and extends the 3' end. The dynamics of the RNA degradation and polymerization reactions of the exosome are not well characterized and thus warrant further investigation in order to better understand the exosome function. Here, we measured the real time activities of the archaeal exosome using single molecule Fluorescence Resonance Energy Transfer (smFRET). Both degradation and polyadenylation activities were highly processive and followed the Michaelis-Menten kinetic parameters over a range of substrate concentrations. Using the combination of smFRET and gel-based assays, we examined these reactions in order to understand the reversibility between 3'→5' nuclease activity and 5'→3' polyadenylation under an equilibrium condition of ADP and P_i. We found that the enzyme can switch the direction rapidly between both reactions back and forth at the condition where both speeds of polymerization and degradation are equivalent.

1887-Plat
Direct Observation of NS3 Substeps at Single Base Pair Resolution
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Helicases are a ubiquitous class of nucleic acid motor proteins. They utilize ATP to catalyze the opening of double-stranded nucleic acids. Previous crystallographic and single molecule fluorescence studies have implied that a single ATP molecule would be used for every base pair (bp) being opened by a helicase. However, this has not been directly observed. The hepatitis C virus encodes NS3, an RNA helicase that is essential for viral RNA replication. Previous single molecule optical tweezers studies on NS3 unwinding have revealed an 11-bp periodicity with 3.6 bp substeps on average. By building an optical tweezers instrument with high spatial resolution and low drift, we were able to monitor the discrete unwinding substeps of NS3 on RNA at single base pair resolution. We show that under conditions where ATP binding limits the motor dwell time between successive substeps, single base pair substeps are clearly visible. We present a distribution of these substep sizes and the implication of this result on mechanisms of NS3 catalyzed RNA base pair opening and strand release. The distribution of motor dwell times between substeps gave us a direct measure of the coupling ratio between ATP and base pair opening, and we propose an integrated model to rationalize all these observations.

1888-Plat
Visualizing an RNA-dependent RNA Polymerase at Work: Polymerization And Strand-switching
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RNA-dependent RNA polymerases (RdRP) are responsible for the transcription and replication of the genomes of many RNA viruses. These enzymes have also emerged as an integral part of the RNA interference mechanism in plants. RdRPs share many of the structural features with other types of polymerases, However, the ability of certain viral RdRPs to perform both replication and transcription sets them apart from more commonly known polymerases such as DNA-dependent RNA polymerases.
We have applied magnetic tweezers to study the transcription kinetics of a model RdRP from Bacteriophage Phi6 (Phi6 RdRP), a double-stranded RNA (dsRNA) virus. During *in vivo* transcription, Phi6 RdRP binds the 3'-end of the antisense RNA strand within the dsRNA genome and polymerases a new sense RNA strand. Concurrently, the original sense RNA strand is displaced. In magnetic tweezers, we measure the transcription kinetics by following the length of the displaced sense RNA strand. As a result, we measure a rate of transcription of ~20 nt/s, which is comparable with our bulk experiments. However, in approximately 30% of transcription events in the magnetic tweezers, we also observe for the first time a conversion of the displaced sense RNA strand back to double-stranded form. By elimination of other possibilities, we attributable this to strand-switching of the RdRP. This is surprising given the 'closed' structure of the RdRP predicted from crystallography. However, it is a phenomenon that has also been observed for DNA helicases and provides a potential mechanism for RNA recombination observed in RNA viruses.

Platform AD: Membrane Receptors & Signal Transduction

1889-Plat
Coarse-grained Modeling And Simulation Of ErbB Receptors: Intramolecular Factors That Govern Outside-in Signaling And Activation
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Epidermal growth factor receptors (ErbB receptors) mediate a number of critical cellular processes including cell proliferation, differentiation, migration and apoptosis. These receptors have received a lot of attention because they have been implicated in a number of human cancers including breast, colo-rectal and prostate cancer. ErbB receptors are also important targets in cancer therapy. The activation and regulation of ErbB receptor function is known to be a highly regulated and multilayered process. A number of elegant structural