explores multiple conformations to find a nucleic acid target. The assay is extended to study the transition mechanism by which RT flips and shuttles on nucleic acids. Distinct effects of increased macromolecular crowding and increased ionic concentrations were exploited to conclude that RT uses tumbling like mechanism to flip ~180° on target site whereas RT uses hopping like mechanism to shuttle during target searching process. We propose that these two distinct mechanisms of RT mobility can be explored for developing new drug targets.

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Nucleotides Linkage Analysis of RecBCD DNA Helicase
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RecBCD is a bacterial enzyme complex that possesses both DNA helicase and nuclelease activities necessary for its biological function. RecBCD is both a continuous and a processive DNA helicase, with an unwinding rate of ~1,600 bp/s. Research on RecBCD function and mechanism in last decades had provided wealth of information on its biochemistry, structure and unwinding mechanism. Still, very little is known about the biochemical transitions, and the nucleotide linkage between RecBCD and DNA during its ATPase cycle. In this work we present thermodynamic linkage analysis using multiple approaches of different nucleotides states of RecBCD free and bound to ssDNA or dsDNA. Primarily, we have purified RecBCD, free of nucleic acid contaminates, which limits such measurements. We show that a nucleotide state mimicking ATP hydrolysis binds with the tightest affinity in comparison to the ADP or nucleotide free binding states in the presence of either ssDNA or dsDNA. Furthermore, by utilizing transient kinetic approaches, we have measured dissociation rate constants of ssDNA and dsDNA from RecBCD with different nucleotides states. We demonstrate that ADP state increases the dissociation rate constant of ssDNA in comparison to all these states: dsDNA-bound, nucleotides free or with the binding of ATP states. Collectively, our results identified a strong nucleotide binding state of the RecBCD complex and a second nucleotide state, which is most likely to dissociate from the DNA track during the course of unwinding and translocation reaction. These results will allow us to construct a model of the different biochemical intermediates during a single ATPase cycle and reveal the allostery that may exist between RecBCD subunits during its unwinding reaction.

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Single-Molecule Studies on DNA Transportation Motors with Common Revolution Mechanism without Rotation
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Nucleic acid translocation is ubiquitous in living systems. The motion required for these events is accomplished by biomotors that hydrolyze ATP. Biomotors were once classified into two categories: linear and rotation motors. Recently, a third class of motor employing a revolution mechanism without rotation was reported. While rotation involves spinning of an object around its own axis, revolution involves the circular movement of an object around a secondary center object. By analogy, rotation resembles the Earth’s motion about its axis once every 24 hours, whereas revolution resembles the Earth ‘circling’ around the Sun once every 365 days. The rotation and revolution mechanisms can be distinguished by the size of channel and the chirality of the channel wall. The channels of rotation motors are equal to or smaller than 2 nm, that is the size of dsDNA, whereas channels of revolution motors are larger than 3 nm. Rotation motors use parallel threads to operate with a right-handed channel, while revolution motors use a left-handed channel to drive the right-handed DNA in an anti-chiral arrangement. This revolving biomotor was found to be widespread among many biological systems, including dsDNA viruses, dsDNA bacteriophages, bacteria, and archaea.

We here present biophysical studies using single-molecule fluorescence and magnetic tweezers, as well as biochemical analysis and structural comparison to illustrate the main features of this newly discovered revolution mechanism on example of the Phii29 bacteriophage DNA packaging motor.

References:

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Single-Molecule Study of Ded1 Helicases using a Hairpin Substrate
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DEAD-box helicases remodel RNA structures, DNA/RNA hybrids and RNA-protein complexes that are essential in gene regulation. So far their mechanism and function is very much debated and the classical or bulk assays are not sufficient to answer these questions. We have developed a new simple magnetic tweezers based single molecule cyclic assay, acting in parallel on tens of molecules at the same time, which allows detecting the unwinding of short RNA/DNA hybrids. Comparing this information with and without an interacting agent can present some insights about the agent. We implement our assay to study DEAD-box helicase Ded1 which is known to only melt a few bases of DNA/ RNA duplex. The observation of unwinding by Ded1 helicase in the presence of ATP indicates that the enzyme melt the duplex rather than translocating along ssDNA. Although DEAD-box proteins are most active with substrates containing single-stranded overhangs, they are also able to unwind short blunt-end duplexes. This has led to the proposal that DEAD-box proteins directly interact with the duplex, but they are “activated” by single-stranded RNA bound at another site. Moreover, it has been proposed that the proteins work by localized destabilization of the RNA helical regions. Hence, the proteins lack processivity. We performed the helicase activity measurement at various concentrations of proteins. Our results show that Ded1 activity is maximum in case of RNA oligo with 5’ overhang. The helicase activity is observed only in presence of ATP while the ATP analogues supported annealing activity. We have also confirmed that the enzyme has no visible effect on the unwinding of DNA.

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Revolution Motors in Cell for Transportation of Lenghty Chromosome without Coiling or Torque
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Double-stranded DNA translocation is essential for biological processes including cell entry, viral genome packaging, cell mitosis, bacterial fission, DNA replication, DNA repair, homologous recombination, and Holliday junction resolution. These motions are accomplished by biomotors utilizing energy from nucleotide hydrolysis. Biomotors were once classified into linear and rotational motors. For a long history, it has been popularly believed that viral dsDNA packaging apparatuses are pentameric rotation motors. Recently, a third class of revolution motor has been discovered (see animations http://nanobio.uky.edu/movie.html#2-7). By analogy, the Earth rotates around its own axis every 24 hours, but revolves around Sun every 365 days. We will present biophysical and biochemical data to elucidate the force generation mechanism of the one-way traffic of revolution motors using phi29 as a model, and how revolution and rotation motors could be simply distinguished by motor channel size and chirality. Due to the length of chromosomes, the induction of DNA supercoiling by biomotors would be a major issue. The revolution mechanism rendered dsDNA void of coiling and torque for translocation of the lengthy helical chromosome, thus with more efficient for the conversion of chemical energy to physical motion.

References

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The Mechanism of Rolling Circle DNA Replication and the Roles of Initiator Protein RepD
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Rolling circle replication is used by plasmids, which carry antibiotic-resistance genes and are transferred between certain bacteria, thereby transmitting the resistance. In this mechanism the two strands are asynchronously copied: one simultaneously with unwinding the parent plasmid, the other strand initially forms a single-stranded circle. As well as polymerase and helicase (PcrA), which are recruited from the host, an initiator protein is also required: RepD in the work described here. This binds specifically to the double-stranded origin of replication and initiates unwinding by nicking one strand and forming a covalent bond with the 5’-end. This nicking opens up a short single-stranded