fibrillation, in support of our REMD simulations. An important finding from our REMD simulations is that fullerene C_{180}, albeit with the same number of carbon atoms as three C_{60} molecules (3C_{60}), and smaller surface area than 3C_{60}, displays an unexpected stronger inhibitory effect on the β-sheet formation. A detailed analysis of the CNP-peptide interaction reveals that strong inhibition of β-sheet formation by CNPs results from the strong hydrophobic and aromatic-stacking interactions between CNPs and β-sheet peptides. These results demonstrate that CNPs can efficiently inhibit the aggregation of β-sheet peptides and reveal the molecular insights into the inhibition mechanisms. Our study reveals the significant inhibitory role of fullerenic hexagonal rings on the aggregation of Aβ (1-22) and full-length Aβ peptides and also provides novel insight into the development of drug candidates against Alzheimer’s disease.

DNA Replication, Recombination, and Repair

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Single Molecule Studies of RPA’s Sequential Binding to ssDNA Reveals a Highly Stiff and Stable State Induced by the Binding of Zinc
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Replication protein A (RPA) is a prototypical modular eukaryotic single-stranded DNA (ssDNA) binding protein that has essential biological function in virtually all DNA transactions in cell. In this work, we used magnetic tweezers to study the sequential engagement of RPA to single large ssDNA molecule in real time. Using mechanical manipulation of single ssDNA tethers, we found that binding of RPA causes ssDNA to rapidly extend and elongate reaching a steady state within several minutes. Analysis of RPA’s effect on the force-extension curve of ssDNA reveals a increased effective persistence length at RPA binding, which indicates a moderate stiffening effect of RPA on ssDNA backbone. Further analysis of RPA’s occupation fraction on ssDNA at varying RPA concentration shows that RPA’s ssDNA binding is largely non-cooperative with an interesting non-monotonic salt concentration dependent binding affinity. Upon examination of the effect of biometals, we discovered a unique role of zinc on RPA’s ssDNA binding: zinc in micromolar concentrations drives formation of a significantly stiffer and more compact RPA coated ssDNA state. These results together provide new mechanoschemical insights to the influences and the mechanisms of action of RPA on large single ssDNA substrate.

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Studies of the FtsK DNA Translocase using Two-Color Tethered Fluorophore Motion
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FtsK actively segregates sister chromosomes during cell division and plays a key role in chromosome maintenance by activating XerCD-dif site specific recombination (SSR). However, important questions remain about FtsK. The mechanism of FtsK assembly at the FtsK orienting polar sequence (KOPS), and the stoichiometry (hexamer vs. dodecamer) of the FtsK translocating unit is unclear. Questions also remain the role of FtsK in biasing SSR to decatenate sister chromosomes, and the mechanics of its activation of recombination. We have recently begun to address such questions using single-color tethered fluorophore motion (TFM), a technique that applies the principles of tethered particle motion using a single fluorophore (rather than a bead); we combined TFM with existing single-molecule methods to observe a new conformational state early in the XerCD SSR pathway and hypothesised that it acts as the substrate for FtsK activation.

Now, here, we present two-color TFM, in combination with Förster resonance energy transfer, using two spectrally separate fluorophores to simultaneously monitor both the DNA conformation and the position of FtsK along its DNA substrate. Our work observes FtsK loading at KOPS and translocating on DNA at 3 kb/s. We show that FtsK dissociates rapidly when it encounters un-synapsed XerCD-dif, but dwells at pre-assembled synapses and activates SSR. After activation, FtsK remains in the vicinity of dif for only ~2 s, and thus cannot decatenate chromosomes by activating multiple rounds of recombination sequentially. Our results also report on the FtsK stoichiometry during translocation. Current work focuses on expanding TFM to a third spectrally distinct fluorophore to elucidate the FtsK structure during translocation and the structural basis for activation of XerCD-dif synapses.

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Unraveling the Interplay between Single-Stranded DNA-Binding Protein, DNA Polymerase and Single-Stranded DNA
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Bacteriophage T7 gene 2.5 protein (gp2.5), a single-stranded DNA binding (SSB) protein, contains two main structural elements which confer it essential functions. First, an oligosaccharide oligonucleotide binding fold which can interact with ssDNA and thus provide a protective role while removing secondary structure impediments. Second, an acidic C-terminal tail capable to interact with other gp2.5 units and with other replisome proteins, such as the T7 DNA primase-helicase (gp4) and polymerase (gp5), allowing for organizational roles within the replisome. The impact of SSBs on the replisome dynamics is hard to study using conventional biochemistry tools. Here, we present data obtained from a unique combination of optical tweezers and confocal fluorescence microscopy, which offers real-time high spatial and temporal resolution to study protein-DNA interactions. Our results show that the SSB gp2.5 binds efficiently to ssDNA, forming highly stable protein-DNA complexes. We also observed a decrease in the ssDNA end-to-end length indicating that the protein binds by wrapping ssDNA, resulting in a highly tension-sensitive binding mode. Using smFRET, we show that the helicase’s binding induces gp2.5 or a mutant lacking the terminal phenylalanine (ΔF), the interaction motif with DNA polymerase, affects replication activity. We show that wt gp2.5 alters the rate, duration and probability of occurrence of replicative and proof-reading polymerase events in contrast to the ΔF mutant or the absence of the protein. Thus, our findings indicate that the presence of gp2.5 strongly stimulates the function of DNA polymerase within the replisome.

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Mechanistic Insights of Hexameric Helicase Function Provided by Single-Molecule FRET
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DNA replication is an essential process across all domains of life. Replicative helicases play an integral part in this process by unwinding the duplex DNA to make single-strand template strands available for duplication. While the steric exclusion model of unwinding, where one strand is encircled by the hexameric helicase and the other excluded from the central channel, is widely accepted, the complexities of this process remain unclear. Details of the helicases’ loading and unwinding mechanism(s) are continually being revealed. One such detail is the interaction of helicase with single-stranded DNA. Our group has recently shown that a wrapping interaction between the excluded single-strand of DNA and the outer surface of the helicase is crucial for the unwinding activity of the 3'-5' MCM helicase from Sulfolobus solfataricus (Sso). Using single-molecule FRET (smFRET), we can now show that this interaction also exists for the hexameric E.coli DnaB (E-DnaB) helicase, which has 5'-3' polarity. The detailed techniques are exhibited by both helicases. This suggests that the interaction may be an important component of hexameric helicase unwinding across various helicase superfamilies independent of polarity. We have also investigated the interaction between E-DnaB’s inner channel and the encircled-strand using smFRET. A compaction of the encircled strand by the helicase has been suggested based on several crystal structures of hexameric helicases bound to single-strand DNA that exhibits a significant decrease in rise per base. Using smFRET, we show that the helicase’s binding induces a ‘scrunching’ of the DNA, and that in the absence of ATP, this is a stable interaction. Both excluded-strand wrapping and encircled-strand scrunching are likely critical aspects of replicative helicase unwinding.

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Effects of DNA Structural and Topological Constraints on HMG2 Binding
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HMG proteins belong to the superfamily of the high mobility group (HMG) proteins that act as architectural transcription factors. They are known to modulate transcription of many genes by altering the chromatin structure. Recently, a new function of HMG2 working as a replication fork chaperone has been uncovered and this finding implies the HMG2 might recognize the particular