Posters: DNA Structure and Dynamics II

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Sequence Selectivity, Cooperativity and Competition in the Equilibrium Binding of Psoralens to DNA

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Psoralens, such as 8-methoxypsoralen, show sequence preference in their photocrosslinking of DNA for TA steps. This uv-induced covalent binding to DNA is preceded by intercalative equilibrium binding. Previously, we have examined the equilibrium binding of intercalators and groove binders using restriction enzyme activity assays. Here we report results for restriction enzyme activity assays of the binding of 5-methoxypsoralen [5-mop] and 8-methoxypsoralen [8-mop] to phiX174 RF DNA [psoralen/DNA base pair from 5.6 - 23]. Eight restriction enzymes possessing differing cleavage sites and differing flanking sequences were employed. The results indicated that binding to flanking sequences affects enzyme activity. For example, both 5-mop and 8-mop cause inhibition of BSSH II [cleavage site GCGCGC]. The structural difference between 5-mop and 8-mop creates differences in binding behavior. 5-mop produces enhanced cleavage by DRA I (TTTAAA), suggesting binding at a site separate from the cleavage site and suggesting that 5-mop produces a DNA conformational change. 8-mop produces inhibition of cleavage, suggesting binding at the cleavage site. Previously we examined selectivity in the binding of DB75, a groove binder [provided by D.W Boykin and W.D. Wilson, Georgia State University]. The presence of DB75 alters the binding of 5-mop near the BSSH II site suggesting possible competition. The presence of DB75 enhanced 5-mop effects near the STU I site [AGGCCT] suggests that, at this locale, the binding sites differ. The studies with combinations of DB75 and either 5-mop or 8-mop provide insight into the activity of these agents separately.

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Using Minicircles to Test the Role of DNA Bending in Mismatch Recognition by Rad4/XPC

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Special proteins involved in cellular processes like gene regulation, replication, and repair bind to specific sites on DNA. Structures of site-specific protein-DNA complexes reveal bent, kinked, or otherwise deformed DNA at the target site, suggesting that proteins distinguish specific from nonspecific sites in part by sensing differences in local DNA deformability. This intrinsic deformability is also influenced by bending and/or topological strain (whether DNA is pre-bent and/or supercoiled). Most studies addressing protein-DNA recognition mechanisms use short, linear DNA that do not reflect the dynamical interactions in the presence of bending/supercoiling, which are expected to have a profound impact on target recognition.Here, we investigate the dynamics of DNA damage recognition by nucleotide excision repair protein Rad4 (yeast XPC ortholog) in the context of DNA minicircles (MCs). Rad4/XPC recognizes a range of helix-destabilizing lesions caused by environmental toxins or UV-radiation. How DNA deformability and the affinity of Rad4 for model lesions are altered when there is bending strain are addressed. We designed ~120-bp MCs containing a CCC/CCC mismatch, previously shown to bind specifically to Rad4. As DNA of these lengths do not bend readily, we employed an innovative approach where two ~60 bp DNA fragments that contained a cognate site for a DNA-bending protein Hbb were pre-bent and then ligated to form MCs with or without mismatches. Competition binding assays revealed that Rad4 binds to both matched and mismatched MCs with significantly higher affinity compared with 24-bp linear DNA with CCC/CCC mismatch. Fluorescence lifetime studies with a cytosine analog FRET pair incorporated in MCs are ongoing to examine how the bending strain introduced affects intrinsic dynamics at the mismatched site compared with a corresponding matched site, and how that correlates with the observed increased binding affinities.

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Parallelized Magnetic Torque Tweezers Probe DNA Mechanics and Viral Integration

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Magnetic tweezers are a powerful tool to probe single DNA or RNA molecules and their complexes with proteins under controlled forces and torques at the single molecule level. We have recently demonstrated several improvements of the magnetic tweezers technique that enable i) fast tracking (~kHz), ii) the capability to directly measure torque, and iii) the ability to perform parallel measurements on many (~100) molecules at the same time. Using these capabilities, we have carried out high-precision measurements of DNA mechanics and dynamics. Our results indicate that the intrinsic torsional stiffness does not change with mono- or divalent ion concentration and is approximately independent of temperature, for temperatures well below the melting temperature. Quantitative comparison of high-resolution single molecules measurements to coarse-grained simulations of DNA mechanics shows that taking into account the anisotropy of DNA and introducing a non-zero twist-bend coupling significantly improves agreement with torque measurements. Further, we demonstrate that all atom molecular dynamics simulations are in quantitative agreement with single-molecule measurements of DNA twist-stretch coupling and correctly predict the temperature-dependence of DNA twist and of DNA torsional stiffness, if the most recent force fields are used. Finally, we report direct measurements of the nucleation of DNA plectonemes that occurs in a force and salt-dependent fashion on a time scale of 10-50 ms. Going beyond bare DNA, we have developed a magnetic tweezers assay to follow retroviral integration in real time, revealing several critical steps along the integration free energy landscape. Our data suggest that integration is driven by a ''downhill'' path in the free energy landscape. In particular, we find an ultra stable strand transfer complex that suggests an important role of resolving factors in vivo.

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The Sequence-Dependent Effects of Branch Migration

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DNA strand displacement is a swapping reaction whereby a single DNA strand invades a partially bound complementary duplex and dislodges a nearly identical strand. Over the past two decades, bioengineers have embraced this reaction as a tool in nanotechnology to create complex structures, logic circuits, and even robots. The mechanism is thought to initiate with the binding of the original single nucleic acid strand to the toehold, the unpaired region of the partial duplex. It has long been thought that the 2 complementary strands compete for base pairs at a junction or ''branch'' which undergoes a one-dimensional random walk until one of the strands is completely displaced. Three-strand branch migration phenomena similarly occur at the heart of central biological systems such as homologous recombination (D-loop) and the CRIPSR/Cas system (R-loop). Despite its wide-ranging importance, the sequence dependent effects of the branch migration step of strand displacement are largely unknown. In this study, we investigate the kinetics of three-strand branch migration and its relation to the sequence of bases occurring early in the branch migration region. We used an innovative single-molecule FRET scheme to allow for direct measurement of branch migration as well as a cost-effective exploration of sequence and length dependence which reveal novel insights into the underlying features of branch migration. We further investigate the effects of salt and temperature. We present a quantitative model to explain the measured waiting time distributions.

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Simultaneous AFM and FLIM Imaging with a SiR-DNA Probe Reveals Structural Changes during DNA Condensation in Live Cell Nuclei

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We show that the use of fluorescence lifetime imaging (FLIM) on the cell-permeable DNA reporter dye SiR-DNA reports on the condensation state of DNA in the nucleus of live cells. As opposed to other probes, SiR-DNA offers the advantages of minimal toxicity, photostability, and the use of gentler far-red light illumination compared to alternative, UV sensitive probes. This permits single photon excitation and compatibility with super-resolution techniques and makes for a powerful probe for live-cell imaging. Using a variety of biological and chemical processes known to induce DNA condensation and structural modifications, we demonstrate that SiR-DNA FLIM informs on state changes in live nuclei without the conventional requirement for cell fixation with alternative probes. We verify our FLIM findings with simultaneous atomic force microscopy (AFM) measurements in the same field-of-view and monitor nuclear stiffness in response to treatments that affect DNA condensation. The acquisition of such multi-parametric biophysical data enables us to highlight and study differences between types of nuclear modifications and their secondary effects.