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Obtaining Quantitative Parameters of DNA-Ligand Cooperative Binding from Persistence Length Measurements

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Binding of ligands to DNA can be studied by measuring the change of the persistence length of the complex formed, in single-molecule assays. We have measured the persistence length of DNA molecule for cationic and neutral beta-cyclodextrin binding, using optical tweezers. We propose a methodology for persistence length data analysis based on a quenched disorder statistical model and describing the binding isotherm by a Hill-type equation. We obtain an expression for the effective persistence length as a function of total ligand concentration, which fits very well our data of the DNA-cationic beta-cyclodextrin and the DNA-HU protein data available in the literature. The fit returns the values of the local persistence lengths, the dissociation constant and the degree of cooperativity for both sets of data. In both cases the persistence length behaves non-monotonically as a function of total ligand concentration. We discuss some physical mechanisms for these binding processes and their interplay with DNA flexibility.

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Probability of Double-Strand Breaks in Genome-Sized DNA by γ-Ray Decreases Markedly as the DNA Concentration Increases

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DNA double-strand breaks (DSBs) present a serious threat to all living things and thus many quantitative studies have been carried out. However, there is no established hypothesis that accounts for the statistics of their production, in particular, the number of DSBs per base pair per unit Gy, P_1, which is the most important parameter for evaluating the degree of risk posed by DSBs. In fact, the reported values scatter by three orders of magnitude [1,2]. This scattering is partly attributable to varying DNA concentrations. So, we evaluate DSBs caused by γ-ray with giant DNA (166 kbp) for a wide region of DNA concentrations using high sensitive single-molecule observation [3]. We find that P_1 is inversely proportional to the concentration above a certain threshold. We give a theoretical interpretation in terms of attack of reactive species upon DNA molecules. Our theoretical model suggests the importance of the size of DNA and its characteristics as semiflexible polymers.

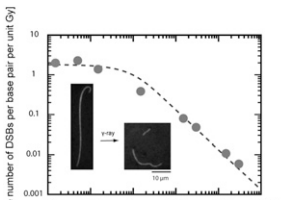

Figure 1: Log-log plot of the number of DSBs per base pair per unit Gy, P_1, as a function of the DNA base-pair concentration, C_{bp}. It is seen that P_1 is roughly constant for small values of C_{bp} and inversely proportional to C_{bp} for large values.

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Probing Single-Molecule Enzymatic Dynamics of B-Glucosidase using Zero-Mode Waveguides

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β-glucosidases (BGLs, EC 3.2.1.21) are exo-type enzymes that hydrolyze β-glucosidic bonds from the non-reducing end of their substrates. BGLs are present in all living organisms (bacteria, archaea, and eukarya), and perform a range of functions. In bacteria and fungi, BGLs play an important role in cellulose saccharification, which catalyzes the hydrolysis of cellobiose and short cellooligosaccharide to glucose. Due to their potential biotechnological importance, a large number of BGLs from bacteria and fungi have been cloned and characterized. Interestingly, these enzymes often exhibit the enzymatic properties such as substrate and product inhibition. Thus, the dynamic molecular properties are too complicated to be fully understood by the use of conventional ensemble techniques. We then employed a single-molecule assay to probe the enzymatic dynamics of BGL.

BGL1B from the wood-rotting basidiomycete Phanerochaete chrysosporium was used in this study. As the enzyme has a relatively low affinity for cellobiose (Km = ~200 μM), μM concentration of fluorescent cellobiose is required to monitor the enzymatic reaction. Therefore, we employed a single-molecule assay using zero-mode waveguides (ZMWs). ZMWs comprise nanoscale holes in an aluminum film deposited on a fused silica coverslip, and can reduce the observation volume by more than three orders of magnitude relative to conventional microscopic techniques, allowing single-molecule observations at μM concentrations of fluorescent molecules in solution. Biotinylated BGL1B was immobilized in ZMWs through a biotin-streptavidin linkage. The BGL1B was immersed in a solution containing 1 μM tetramethlyrhodamine-conjugated cellobiose (TMR-cellobiose). The repeated appearance and disappearance of TMR fluorescence were often observed, indicating that immobilized BGL1B hydrolyzes TMR-cellobiose in ZMWs. Surprisingly, we have found that the enzymatic reaction is inhibited by glucose non-competitively at the lower concentration and competitively at the higher concentration.

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DNA Dynamics under Nano-Confinement

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DNA polymer dynamics are fundamental to the function of biological systems. Examples include gene regulation, cell division, threading and transport through pores. We studied the dynamics of DNA molecules ranging in length from 40bp to 20kbp both in solution and under confinement in nano-slits. DNA molecules were 2-color end labeled and their internal polymer dynamics were observed by fluorescence microscopy and fluorescence cross correlation spectroscopy. We also present a modified fitting model for FCCS results taking into account confocal volume overlap imperfection and nano-slit confinement. We experimentally determined the diffusion coefficients of a range of different weight DNA molecules. Better understanding internal polymer dynamics under nano-confinement has potential applications in genomic sequencing, single biomolecule manipulation, and separations as well as the ability to address fundamental research questions in biophysics and molecular biology.

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Single-Stranded DNA Curtains for Real-Time Single-Molecule Visualization of Protein–Nucleic Acid Interactions

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Single-molecule techniques have greatly advanced our understanding of many types of biochemical reactions. We have established strategies for anchoring and organizing 'double-stranded' DNA (dsDNA) molecules on the surfaces of microfluidic sample chambers that are coated with a fluid lipid bilayer. This technique called ''DNA Curtains'' has proved powerful in the examination of proteins as they bind, diffuse and translocate along dsDNA. Single-stranded DNA (ssDNA) is a crucial intermediate in nearly all biochemical reactions related to the maintenance of genome integrity. However, most single molecule studies of proteins on ssDNA use short synthetic oligonucleotide substrates. Here, we present procedures for generating, aligning and visualizing hundreds of long single-stranded DNA molecules along the leading edges of nanofabricated barriers, where the DNA can either be ''single-tethered'' or ''double-tethered''. This new approach permits long-desired access to critical biological reactions involving single-stranded DNA binding proteins.

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Single-Molecule Studies of Adenovirus Maturation

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Instead of relying only on three-dimensional diffusion to associate with a target on DNA, many DNA-binding proteins reduce the dimensionality of search by transiently diffusing along DNA and thus speed up the recognition process. Previously, it has been shown that during the maturation of a single adenovirus particle ~70 copies of the adenovirus protease (AVP) have to cleave ~3200 target proteins situated on the viral DNA in order to render virus particles