973-Plat
High Flexibility of Short DNA Probed by Single Molecule Cyclization
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DNA duplex is classically viewed as an elastic rod and its mechanical properties are described by the worm-like chain model (WLC) with a persistence length of around 50 nm or 150 basepairs. However many biologically significant protein-DNA interactions involve looping and bending of DNA on a shorter length scale. Despite its conceptual and practical importance, experiments and computer simulations to measure cyclization of sub-persistence DNA molecules have yielded contradictory results. We developed a fluorescence-based cyclization assay for directly observing the cyclization of single DNA molecules. Our results show that short DNA of lengths 67- to 105-bp have an effective j-factor of up to 10^{10} times higher than the theoretical predictions. Our results also show that DNA at this length scale is more than 1000 fold more twistable than the model prediction. Finally we propose to use this assay as a fast and versatile tool to study the effect of DNA binding proteins and ligands as well as DNA defects on the global cyclization of DNA.

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Single Molecule Studies of the c-di-GMP Riboswitch
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Cyclic diguanylate (c-di-GMP) is a second messenger signaling molecule important for the regulation of many bacterial processes, including virulence, motility, and biofilm formation. Recent developments have shown that c-di-GMP is recognized by a riboswitch, an mRNA domain that controls gene expression in response to varying concentrations of a target ligand. The c-di-GMP riboswitch has been shown to undergo a global structural rearrangement in response to ligand binding. We have used single molecule fluorescence resonance energy transfer (smFRET) to investigate this conformational change. In the absence of c-di-GMP, the riboswitch exists in primarily an undocked conformation and only in the presence of the correct substrate can it fold into a stable docked conformation. This docked conformation was found to be stable for at least 30 minutes. As this riboswitch is responsible for the regulation of many critical bacterial processes, the stability of the docked conformation in the presence of c-di-GMP may be important to ensure a constant expression of required genes. In the presence of c-di-AMP, the riboswitch displayed conformational behavior similar to that in the absence of substrate, demonstrating the specificity of this riboswitch. A population of dynamic molecules that exists primarily in a folded conformation was found to increase in the presence of high magnesium concentrations leading to the idea that magnesium ions aid in the conformational switch from an undocked to folded conformation but are not able to fully stabilize the docked conformation.

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The Conformational Dynamics of Model Slipped Strand DNA Structures are Determined by Base Pairing in the Heteroduplex Arm
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Slipped strand DNA is formed from the out-of-register pairing of direct repeats into thermodynamically stable hairpin structures. The formation of slipped strand DNA is hypothesized to be an important intermediate in triplet repeat expansion, a mechanism associated with many hereditary and anticipative neurodegenerative diseases. In this study, we use three-way DNA junctions as models for slipped strand DNA structures. Each of the three-way DNA molecules have two homoduplex arms and one heteroduplex arm, and the structures and conformational dynamics of the model substrates are characterized using single-molecule fluorescence resonance energy transfer (smFRET). FRET can measure changes in the relative distances between two fluorophores that are sufficiently close together (20-80 Å). In our experiments, junctions are end-labeled with donor and acceptor fluorophores, and the efficiency of energy transfer E between the fluorophores is measured as a function of time to approximate the distances between the fluorophores and to monitor the conformational dynamics of the arms. We find that the junctions have trigonal pyramidal geometry. In addition, we observe that the junctions with two or more A-A mispairs on the heteroduplex arm transition between two conformational states with conformer transition rates that are dependent on ionic strength. This investigation sheds light on the structural and dynamical properties of junctions formed by slipped strand DNA which are important for understanding how enzymes recognize and process these structures.

PLATFORM U: Membrane Receptors & Signal Transduction II

976-Plat
Design and Application of a Light-Activated Metabotropic Glutamate Receptor for Optical Control of Intracellular Signaling Pathways
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The ability to manipulate ion channels with light via photoisomerizable tethered ligands (PTLs) has opened the door for the optical control of neural activity both in cultured cells and *in vivo*. We have extended this to G-protein-coupled receptors by making a light-activated metabotropic glutamate receptor (mGluR). Taking into account pharmacological, structural and computational data, we screened PTLs in the MAG (Maleimide-Azobenzene-Glutamate) family, which included previously synthesized L and newly synthesized D stereoisomers with variable linker lengths. These were tested at several different sites of cysteine introduction in the ligand binding domain of mGluR2. Photo-control was tested by patch clamp recordings of HEK293 cells expressing the cysteine-substituted mGluR2 along with a G-protein-coupled inward rectifier potassium channel (GIRK) as a reporter. We identified combinations of cysteine substitutions, MAG stereochemistry and linker length that worked as either a light-gated agonist or antagonist of mGluR2. Liganding was reversible, turned on by 380 nm light that photoisomerizes the azobenzene to *cis* and turned off by 500 nm light that photoisomerizes the azobenzene to *trans*. The efficacy and kinetics of photo-activation and antagonism approached those obtained with pharmacological agents. In cultured hippocampal neurons the light-activated mGluR2 activated GIRKs, and hence rapidly and reversibly suppressed excitability. In hippocampal autapses the light-activated mGluR2 inhibited both glutamatergic and GABAergic synaptic transmission and altered short-term plasticity with equal reversibility and speed. The ability to optically manipulate mGluRs provides an orthogonal, non-invasive approach to probe the role of intracellular signaling pathways in biological functions with high spatiotemporal precision. Future work aims to use these optogenetic tools to probe the spatiotemporal properties of mGluR-mediated forms of long-term plasticity in intact brain slices and *in vivo*.

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Homo- and Hetero-FRET Imaging of Membrane Proteins in Live Cells
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The structure-function-activity relationships of transmembrane receptors are often mediated not only by ligand-induced signaling but also homo- and hetero-philic binding interactions. Understanding the molecular basis for these interactions is therefore critical for elucidating receptor function. We are using live cell total internal reflection fluorescence microscopy (TIRFM) to examine the distribution, association, and ligand accessibility of (1) carcinoembryonic antigen-related cell-adhesion molecule 1 (CEACAM1), and (2) fibroblast growth factor receptor 1 (FGFR1), which is thought to associate with FGF21 co-receptor Klotho-beta (KLB). By combining TIRFM with homo- and hetero- Förster Resonance Energy Transfer (homo-FRET and hetero-FRET, respectively), we are able to track the real-time response of CEACAM1 oligomerization and dynamics to soluble factors (such as CEACAM-specific antibody, ionomycin, and soluble CEACAM1), as well as KLB's and FGFR1's responses to FGF21. Obtaining a better understanding of the molecular dynamics of CEACAM1, KLB, and FGFR1 as well as their spatial and oligomeric distributions is important for elucidating their roles in downstream signaling pathways.

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Molecular Mechanisms of T Cell Signal Transduction Revealed by Super-Resolution Fluorescence Microscopy
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We employ single molecule imaging techniques to understand how activation of the T cell receptor on the cell surface leads to an intracellular signaling response. To function in an immune response, T cells become activates when