1478-Pos Board B322
Use Of Single-Molecule Imaging To Analyze The Distribution Of Binding Ability In RNA Aptamer Populations
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The number of possible sequences for nucleic acid and protein biopolymers of functionally plausible lengths is literally beyond astronomical, exceeding the probable number of atoms in the universe. The nature of the functionality landscape across sequence space for such molecules is of great interest to the organism of life field, and, more pragmatically, to those interested in the design, screening, or in vitro evolution of functionally useful molecules. This encompasses many questions relating characteristics of such molecules, such as stability or structural motifs, to the distribution of ability, as measured by kinetic rates. A key question concerns the likelihood of finding particular functional abilities (binding or catalysis) in a pool of sequences with a given length and/or other complexity-determining attributes.

Investigating the “kinetic structure” of a population is not possible with conventional bulk methods as subpopulations are simply averaged together. Such fine structure can only be approached using single-molecule techniques. This project uses a single-molecule fluorescence microscopy technique (Total Internal Reflection, or TIR) to analyze binding kinetics in populations of RNA aptamers. RNA is a particularly good candidate for exploration, owing both to its centrality in the RNA world hypothesis and the current interest in developing RNA aptamer based drugs.

Experiments with known GTP aptamers yield on and off rates that differ by species, are comparable to bulk results, and enable species separability in kinetic space. The goal is to enable quantification of the distribution of binding ability in heterogeneous, high complexity pools. In addition to addressing questions related to functional RNA, this is useful for designing and understanding in vitro selection experiments, a key tool for the origins of life field and the expanding field of applied molecular evolution.

1479-Pos Board B323
Sensitivity Of DNA-hairpins Dynamics To The Mechanism Of Force Feedback Probed Using A Surface-coupled Passive Force Clamp
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Optical-trapping experiments have yielded new insight into the mechanical behavior of individual biomolecules. A common experimental assay consists of an enzyme or nucleic acid molecule attached to a cover slip at one end and to a small polystyrene bead at the other. The bead is captured and held under tension with an optical trap. For this reason, it is useful for designing and understanding DNA under a temperature-controlled microscope (50~70°C). Here we show the

-1480-Pos Board B324
Proof of Principle for Shotgun DNA Mapping by Unzipping
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We are developing single-molecule methods for mapping protein-DNA interactions inside living cells by unzipping single chromatin fragments isolated from living cells. One avenue towards this capability involves unzipping random fragments that have been generated by site-specific restriction endonuclease digestion of whole genomic DNA or chromatin, a process we are calling shotgun DNA mapping or shotgun chromatin mapping. A key enabler of shotgun DNA mapping (SDM) will be the ability to assign the individual fragments to their specific sites in the genome, based on the sequence-dependent unzipping force of the underlying naked DNA sequence. We will present proof-of-principle results demonstrating the ability to match experimental data sets for pBR322 unzipping to the correct pBR322 sequence hidden in a library of approximately 3,000 yeast genome sequences arising from the known locations of XhoI recognition sites. We do so via an algorithm that scores the experimental data against simulated unzipping forces from a quasi-equilibrium model (Bockelmann, Essevaz-Roulet, & Heslot, 1997).

Our next step is to perform SDM on yeast genomic DNA fragments produced by ligation of XhoI-digested DNA to unzipping constructs. Enhancements of the matching algorithm, data processing, and unzipping simulation will be discussed, along with studies of the robustness of the SDM method as a function of number of sites in genome and other parameters. In addition to the impact on our goal of single-molecule mapping of chromatin from living cells, SDM may have important applications in other areas of genomics, including high-throughput structural DNA mapping and genome-wide mapping of sequence-specific DNA binding proteins.