on polymerase binding to chromosomal DNA, whereas FRET efficiencies report on protein conformational states (E*~50%: binary polymerase-DNA complex; E*~70%: ternary polymerase-DNA-nucleotide complex). These novel and general smFRET tools should allow visualization of protein structure *in vivo* and report on how conformational changes affect cellular mechanisms.

1131-Plat
Single Molecule Diagnostic Method to Reveal Cancer-Related EGFR Signaling
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Receptor tyrosine kinases (RTK) regulate cell fate such as differentiation, proliferation, survival and migration via recruiting SH2 containing downstream proteins. Dysregulated interaction between RTK and SH2 containing downstream protein incessantly activates MAPK or PI3K-Akt pathway which will cause a cancer in various organs. Real-time single-molecule co-immunoprecipitation (co-IP) is able to reveal quantitatively these protein-protein interactions using endogenous proteins at single molecule resolution. Here we develop a single molecule diagnostic method measuring endogenous epidermal growth factor receptor (EGFR) signaling extracted from cancer cell lines or lung tissues of cancer patients with eGFP labeled Grb2 as a probe, an adaptor protein containing SH2 involved in various EGFR signaling pathways using real-time single-molecule co-IP. This method distinguishes cell lines or tissues expressing highly activated EGFR. We also demonstrate that even cell lines have the same EGFR mutation, αA746-E750, protein interaction network can be altered, which is not revealed by genome sequencing based diagnosis. Our approach allows us to investigate signaling network of proteins at single molecule resolution. It also suggests a concept of a molecular diagnostic method at protein-protein interaction level.

1132-Plat
Improved Single-Molecule Force Spectroscopy Using Micro-Machined Cantilevers
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Enhancing the short-term force precision of atomic force microscopy (AFM) while maintaining its long-term force stability shows promise for improving AFM performance across multiple modalities, particularly single molecule force spectroscopy (SMFS). SMFS is a powerful method to probe the dynamics and energetics of a wide range of bio-molecules (proteins, RNA, and DNA). The equilibrium folding and unfolding of such macromolecules is sensitive to sub-pN changes in force. Recently, we demonstrated sub-pN force precision and stability over a broad bandwidth ($\Delta f = 0.01$-20 Hz) by removing a cantilever's gold coating. Maintaining long-term force stability requires soft cantilevers, due to instrumental noise in cantilever detection at low frequencies. Improving short-term force precision requires decreased hydrodynamic drag, a consequence of the fluctuation-dissipation theorem. We met these two - often competing - goals by using a focused ion-beam to micromachine a short ($L = 40$ μm) commercial cantilever. Our efficient process led to a 10-fold reduction in stiffness and a 10-fold reduction in the effective hydrodynamic drag at affordable cost (~$30/cantilever in an academic setting). As a result, we extended the AFM's sub-pN bandwidth by a factor of ~50 to span five decades of bandwidth ($\Delta f =$ 0.01-1,000 Hz). Moreover, we did so while preserving a cantilever's high reflectivity while avoiding the detrimental effects of a gold coating. Finally, the benefits of micromachined cantilevers were demonstrated by mechanically unfolding a polyprotein, a common substrate for SMFS experiments. We expect these responsive yet stable cantilevers to broadly benefit AFM-based research.

1133-Plat
Fast Spatiotemporal Correlation Spectroscopy to Determine Protein Lateral Diffusion Laws in Live Cell Membranes
Carmine Di Rienzo[1], Enrico Gratton[2], Fabio Beltram[1], **Francesco Cardarelli**[3].
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Here we present a straightforward image correlation analysis method to study the dynamics of fluorescently-labeled plasma-membrane proteins in live cells with high spatiotemporal resolution1. Notably, we don't extract and track each molecule, but we calculate population behavior using all molecules in a given region of the membrane. First, fast imaging of a given region on the membrane is achieved. Then, acquisitions at increasing time delays are correlated, for example each 2, 3, n repetitions. If particles diffuse, the width of the peak of the spatial autocorrelation function increases as the time delay between frames increases. Fitting of the series of autocorrelation functions enables to extract the actual protein 'diffusion law' from imaging, in the form of a mean square displacement vs time-delay plot (iMSD). The iMSD yields a quantitative view of the temporal evolution of the average molecular positions with nanometer accuracy, and no need for interpretative models. We demonstrate the potentiality of our approach by studying the regulation of protein lateral diffusion in live cell membranes. By using a GFP-tagged variant of the Transferrin Receptor (TfR) we are able to observe the regulation of protein diffusion imparted by the cytoskeleton meshwork on μm-sized membrane regions in the micro-to-milli-second time range. We show that our approach can successfully recover TfR diffusion parameters over many microns, and their variation in response to drug treatments or temperature shifts. Potential extension of this method to the 3D intracellular environment and differences with respect to other approaches will be discussed.
1. Di Rienzo, C., Gratton, E., Beltram, F. & Cardarelli, F. Proc Natl Acad Sci USA 110, 12307-12 (2013).
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1134-Plat
DNA Y Structure: A Multidimensional Single Molecule Assay
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Single molecule techniques have enabled significant advancement in the understanding of biological systems. However, current optical trapping techniques allow for force, extension and torque to be measured only along the axis of applied tension. This one dimensional aspect limits studies of complex biological systems due to the difficulty of data interpretation. Next generation optical trapping assays need to expand the number of measurement dimensions to capture the behaviors of multi-component biological systems. In addition, it would be advantageous for these new assays to combine optical trapping with fluorescence. Here, we present a novel assay which utilizes a dual beam optical trap to hold a three-arm DNA construct, which we call a Y structure. This design combines all manipulation capabilities of existing optical trapping techniques and enables simultaneous stretching, unzipping, and twisting of the same piece of DNA. We have characterized the mechanical properties of the Y structure by unzipping under force and torque, and have theoretically modeled the resulting data. We have also demonstrated that this assay is compatible with fluorescence by unzipping through a fluorescently labeled DNA-bound protein and observing its subsequent fate. These features, taken together, should enable the study of more complex biological systems.

Platform: Micro- and Nanotechnology I

1135-Plat
Regulating Spatiotemporal Dynamics of Notch Signaling in Live Cells via Magnetoplasmonic Nanoprobes
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Notch signaling is a key cell-to-cell communication mechanism during development and in normal tissue maintenance and cancer. Signals exchanged between neighboring cells via Notch can reinforce molecular differences which eventually direct the fate decision of individual cells. Despite increasing knowledge of these signaling events, little is known about how spatiotemporal dynamics of the receptor signaling across a cell influence the signal exchange. In this presentation, we introduce an advanced nano-probing system that mimics binary cell communication via Notch, while providing systematic spatiotemporal control of Notch signaling in live cells. This new nanosystem enabled simultaneous observation of Notch dynamics and signal activation with single molecule resolution in live cells for the first time. We envision this nano-probing system as a next generation force microscopy technology platform to quantify and control force-mediated biological processes at the subcellular level.