Multiple calibration techniques were used to verify the force range of ~0.01 to ~ 100pN and the accuracy of extension measurements obtained in image processing. A micro-spray technique has been developed to introduce protein solutions to the taut, single DNA construct to observe individual protein binding events. Using these techniques we will evaluate the binding energetics of nucleosome formation with modified histones at a single molecule level.

832-Pos Board B632
Mechanically Unzipping Double-Stranded DNA with Built-In Sequence Inhomogeneities and Bound Proteins
Ping Lu, Abhijit Sarkar.
We theoretically analyze the force signal expected during unzipping of DNA with a) bound proteins and b) when the DNA is more strongly base-paired over certain regions. We consider the case of a single bound protein, multiple isolated bound proteins, bound proteins with cooperative interactions that result in collective force-induced unbinding events, and the case of a very large number of bound proteins. In addition, we also analyze the case where the unzipping proceeds through multiple, isolated DNA regions which are more strongly bound than the surrounding DNA. Our calculations are done in the fixed-extension ensemble. In both cases we find two different types of force traces which we label sawtooth profile and ramp-plateau profile.. In the former, the force extension curve has a series of sawtooth peaks superposed on the usual constant force of ~15pN found unzipping bare DNA, while in the ramp-plateau case, the force near an unzipping constraint increases roughly linearly and then levels off and then returns to its baseline value (~15pN) after the protein in unbound or the strongly base-paired region is disrupted allowing the unzipping fork to pass through. These shapes are correlated with the positions of bound proteins or sequence inhomogeneities. We calculate how the force-extension profile depends on the protein or sequence parameters. Our results compare well with observed in unzipping of natural-sequence DNA and DNA with bound proteins. These results point the way toward inferring sequence-related information and protein binding enthalpies from single molecule unzipping experiments.

833-Pos Board B633
High-Throughput Single Molecule Measurements Confirm a Load-Dependent Association Rate for E-Selectin and Sialyl Lewisa
Jeremy H. Snook, Alexander R. McClure, William H. Guilford.
We previously reported the association rate between E-selectin and sialyl Lewis[a], two molecules involved in leukocyte adhesion and rolling, to be accelerated by compressive loads acting between them (Snook and Guilford, 2010). To improve and expand these studies, we have built a magnetic bond pulling system for high-throughput data collection from single molecules for studies of load-dependent kinetics. The system allows us to replicate laser trap and biomembrane force probe experiments on a larger scale. Four electromagnets are arranged in a tetrahedron, two above and two below a flow cell. These provide uniform upward and downward forces, respectively. The system is controlled through a LabVIEW interface and is capable of producing vertical pushing and pulling forces up to ~40 pN between receptor-coated surfaces and 3 μm ligand-coated superparamagnetic beads. Loads are applied to dozens of beads simultaneously, which are tracked through video microscopy with a temporal resolution of 11 ms. We used the device to investigate the possibility of a load-dependent rate of association between E-selectin and sialyl Lewis[a]. The fraction of contacts forming a bond (''adhesion probability'') can be fitted as a function of contact time to determine the 2-dimensional on-rate. This was done over a range of compressive forces to determine the effect of compressive force on 2D on-rate. We confirmed that compressive force does indeed increase the rate of association between E-selectin and sialyl Lewis[a]. This high-throughput approach will allow us to more rapidly investigate the origin of this phenomenon.

834-Pos Board B634
Passive Torque Wrench and Angular Position Detection Using a Single Beam Optical Trap
James Inman, Scott Forth, Michelle D. Wang.
The recent advent of angular optical trapping techniques has allowed for rotational control and direct torque measurement on biological substrates. Here we present a novel method that increases the versatility and flexibility of these techniques. We demonstrate that a single beam with a rapidly rotating linear polarization can be utilized to apply a constant controllable torque to a trapped particle without active feedback while simultaneously measuring the particle's angular position. In addition, this device can rapidly switch between a torque wrench and an angular trap. These features should make possible torsional measurements across a wide range of biological systems.

835-Pos Board B635
Spatial Distribution and Dynamics of Syntaxin-1 in Live PC-12 Cells
James C. Weisshaar, Renee Dalrymple.
The soluble N-ethyl-maleimide-sensitive factor-attachment protein receptor (SNARE) proteins play an important role in vesicle fusion. The spatial distribution of these proteins in live cells is largely unknown. Clusters of syntaxin-1 (Syx-1) were observed previously by Hell, Lang and co-workers using stimulated emission depletion (STED) on PC-12 cell membrane sheets with immunostaining.[1] The clusters may be docking sites for large dense-core secretory vesicles (LDCVs), which can then undergo exocytosis. In this study we transfected rat pheochromocytoma (PC-12) cells with a plasmid encoding Syx-1 tagged with the photoactivatable protein mEos2 at the N-terminus. Photoactivation and localization of single molecules was used to study the distribution of Syx-1 in live PC12 cells, while single particle tracking (SPT) was used to study the dynamics. The data indicate inhomogeneous diffusion, with some Syx-1 molecules appearing confined and others seemingly diffusing freely. A plot of is initially linear with a slope corresponding to a free diffusion constant of ~0.15 μm^2/sec. The spatial distribution is somewhat clustered, but seemingly much less so than in the earlier study of unroofed cells. LDCVs were imaged in sequence with Syx-1 using EGFP labeled tissue plasminogen activator (tPA). Preliminary data suggest that LDVCs visit the plasma membrane along specific branched paths whose locations tend to be anti-correlated with the distribution of Syx-1.
[1]**JJ Sieber, *et al. Science 317*, 1072–1076 (2007).**

836-Pos Board B636
Single-Molecule Fluorescence Study on Membrane Proteins Derived from Living Organisms: Application to Drosophila Olfactory Receptor Or83b
Hong-Won Lee, Han-Ki Lee, Si-On Lee, Kipom Kim, Du-Young Min, Walton Jones, Tae-Young Yoon.
Or83b is a broadly expressed receptor protein for *Drosophila melanogaster* olfaction, but its exact function has not yet been identified. It is still sharply debated whether Or83b is a simple signal mediator in cooperation with other receptors or Or83b itself acts as a part of a cation channel. Here, we describe a general methodology for investigating the function of membrane proteins, derived from living organisms, using single-molecule fluorescence techniques. We reconstituted Or83b and other membrane proteins from *Drosophila* antennae in 50-nm sized vesicles. Then, total internal reflection fluorescence (TIRF) microscopy was used to reveal the stoichiometry of Or83b reconstituted in vesicles. The functionality of Or83b was checked by fluorometric analysis that detected Ca^{2+} ion influx into the proteoliposomes, which could be triggered by addition of odorant molecules. Our experiment may provide a general methodology for studying various membrane proteins, which do not have available recombinant forms.

837-Pos Board B637
Single Molecule TIRF Study of Fibrinogen Polymerization
Alina L. Popescu, Kathryn K. Gersh, Dan Safer, John W. Weisel.
We used the bleaching behavior of fluorescently labeled fibrinogen molecules in total internal reflective fluorescence microscopy (TIRF) together with calculations of labeling probability to determine the number of active fluorophores attached nonspecifically to a fibrinogen molecule. From the total intensity of the bleaching steps - as single molecule signature events - and the probability of active labeling of the molecules, we obtained a single molecule intensity calibration. Fibrin fibers were observed and characterized in TIRF by using this calibration. Fibrinogen polymerization was induced in the TIRF observation chamber by diffusive mixing of fibrinogen and thrombin, which initiates polymerization by cleavage of the fibrinopeptides to produce fibrin. We show that live observation of fibrin fiber growth in TIRF can be translated by this calibration in molecular information on the growth kinetics and molecular mechanisms of fibrin fibers formation.

838-Pos Board B638
Imaging an Expanding Molecular Robot World Using Super-Accuracy Single-Molecule Fluorescence Microscopy
Nicole Michelotti, L. Devon Triplett, Alex Johnson-Buck, Anthony J. Manzo, Jeanette Nangreave, Steven Taylor, Milan N. Stojanovic, Hao Yan, Nils G. Walter.
We recently demonstrated the concept of molecular robotics with a synthetic DNA-based nanowalker, dubbed a ''spider'' composed of a streptavidin protein "body" attached to three biotinylated DNA enzyme legs, along a one-dimensional track of chimeric DNA-RNA substrates positioned on a DNA origami (1,2). By cleaving its substrates, the spider weakens the binding energy between its legs and previously visited sites, resulting in a biased, processive, random walk towards fresh substrate. Additional components are now being incorporated into the spider world to increase its versatility and complexity in behavior. For example, we are implementing a second spider that walks on a different substrate. This spider can