Intracellular Ca2+ signaling has a central role in regulation of salivary gland cell function. Coordination of Ca2+ signaling between cells contributes to synchronized and effective secretion of saliva. However, mechanisms that underlie this signaling remain elusive. Here, intercellular Ca2+ waves (ICW) and their propagation in human salivary gland (HSG) cells were investigated using fura-2 fluorescence imaging. While not well understood, mechanical stimulation of a single cell in a cluster with a micropipette induces ICW. The Ca2+ signal is propagated from the stimulated cell to the 7-9th tier of cells or ~120 μm. The following findings indicate that ICW propagation in HSG cells uses an extracellular and ATP-dependent pathway. The purinergic receptor antagonist suramin significantly decreased ICW propagation. Extracellular ATP or UTP abolished ICW suggestive of receptor desensitization. Gap junction intercellular communication is not involved in ICW in HSG cells because the gap junction inhibitor oleamide did not inhibit ICW. Furthermore, HSG cells showed poor dye coupling upon microinjection of Lucifer Yellow. The Ca2+ transients observed within each cell are dependent on Ca2+ release from the ER as thapsigargin abolished the ICW. The phospholipase C inhibitor U73122 also blocks ICW indicating that these transients are IP3-dependent. Furthermore, store operated Ca2+ entry (SOCE) modulates the amplitude of Ca2+ signal since removal of extracellular Ca2+ or a SOCE inhibitor SK&F 96365 decreased the amplitude of Ca2+ signal. Inhibition of mitochondrial Ca2+ uptake with FCCP/oligomycin or ruthenium red showed similar effects on the amplitude. These results indicate that propagation of this ICW utilizes extracellular ATP, likely through the P2U(P2Y2) receptor in HSG cells. The major Ca2+ mobilization mechanisms are IP3-dependent ER Ca2+ release and SOCE. Finally, mitochondrial energy metabolism and Ca2+ uptake modulated this ICW propagation.

Emerging Single Molecule Techniques II

1463-Pos Board B307

Distortion of Protein Receptor Decreases the Lifetime of Receptor-ligand Bond

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Ligand competition assay is often used in single-molecule force spectroscopy (SMFS) to test the specificity of binding. We have noticed that in the SMFS measurements that utilize biotin tethered to the tip of an atomic force microscope and streptavidin bound to the surface, addition of ~1mM of free biotin in solution does not completely eliminate binding events as detected by SMFS. We hypothesize that the compressive force applied to the streptavidin-biotin complex on the substrate during the measurements shortens the bond lifetime. We have tested this hypothesis by performing a series of measurements with different maximum compressive force applied to the surface. These measurements indicate that the compressive force affects the number of interactions measured in the presence of free biotin. The measured dependence agrees with the model that takes into account the increase of the tip-surface contact area with an increase of the maximum applied force. These results indicate that for SMFS to be used as a competition assays, shortening of a lifetime of the receptor-ligand bond by compressive force should be considered.

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Immobilization of Single Biomolecules Using Covalent-Bond Linkages for Fluorescence Single-Molecule Experiments

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The streptavidin-biotin bridge is commonly used in single-molecule studies to surface immobilize biomolecules onto microscope slides. However, the presence of tryptophanes impedes utilization of UV light and numerous fluorescent nucleotide analogs, such as 2-aminopurine. We are developing new approaches to immobilize DNA/RNA molecules without use of streptavidin and biotin. One approach consists of using the Huisgen cycloaddition reaction between an alkyne and an azide, which is an example of ''click'' chemistry reaction. In this ''click'' chemistry approach, 3′-azide modified oligos are immobilized to an alkyne-modified microscope slide surface through a triazole linkage. This cycloaddition reaction is very stable in many physiologically relevant buffers, and has been shown to occur without the need of a catalyst. In another approach, we take advantage of the efficient coupling between thiol groups to immobilize biomolecules by forming disulfide bridges. 3′-thiol modified oligos are surface immobilized on a thiol-modified microscope slide by forming disulfide bonds. We are currently improving the immobilization efficiency by optimizing the reaction parameters and conditions. We anticipate that these approaches will allow us to investigate local conformational changes in biomolecular systems at the single molecule level.

1465-Pos Board B309

A Flexible Anti-Brownian Electrokinetic (ABEL) Trap for Single-Molecule Immobilization in Solution

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We demonstrate our ability to trap and probe individual fluorescent particles in solution using an improved anti-Brownian electrokinetic (ABEL) trap. Traditional single-molecule immobilization techniques include surface attachment and laser tweezers; the former technique often disrupts fragile biochemical systems, while the latter requires that molecules be conjugated to large beads. The ABEL trap circumvents these issues by tracking the motion of a particle via fluorescence, and applying electrokinetic feedback forces to cancel its Brownian motion.

Our ABEL trap suppresses the Brownian motion of a fluorescent particle as follows. A laser beam is rapidly steered in a small scan pattern near the center of a microfluidic cell. An avalanche photodiode detects fluorescence photons from the molecule. A field-programmable gate array compares the precise arrival time of each photon with the known position of the laser, and generates a corresponding feedback voltage. The feedback voltages is amplified and applied to the trap. We use a broadband supercontinuum laser with an acousto-optic tunable filter to enable fluorescent tracking in any part of the visible spectrum, and we scan the laser using electro-optic deflectors that can function at up to 100 kHz. This combination of hardware enables precise spatial, temporal, and spectral control of our illumination and detection optics and can apply feedback at a latency of 2 μs, a better-than-tenfold improvement over previous trap designs. We hope that these improvements will enable us to trap single small-molecule fluorophores in solution.

The flexibility of the ABEL trap makes it amenable for a wide variety of biophysical studies. Work is currently underway to apply the ABEL trap to study the dynamics of DNA in solution. In the future, we hope to apply the trap to study the kinetics of proteins such as proteorhodopsin.

1466-Pos Board B310

Automating Optical Tweezers Experiments With a Microfluidic Laminar Flow Channel Device

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Optical tweezers perform in singulo experiments on biological reactions that occur stochastically, often through multiple pathways. Characterization of single molecule trajectories allows determination of conformational distributions and detection of intermediates. However, this approach requires repeating the measurements tens or hundreds of times to achieve sufficient statistics. Aiming for high-throughput experiments, we combine microfluidic delivery of beads into the assay chamber with automated optical tweezers.

We have developed a computer controlled microfluidic device for nano-litre sample-handling. Feedback control is achieved by monitoring and setting the pressure differences between individual inlet reservoirs and the outlet with high (<1 Pa, ca. 0.1 mmH2O) precision. This allowed us to achieve stable, repeatable, fluid flow in the micron-sized channels of a typical lab-on-chip setup. As a proof-of-principle experiment we performed repeated force-extension measurements on ~10 kb dsDNA-molecules. Preliminary results on automatic assembly of the dumb-bell assay (bead-DNA-bead construct) and force-extension measurements will be presented. These automated, high-throughput, single-molecule experiments allow us to study rare events and phenomena in nanoscale biological physics, often inaccessible to other methods.

1467-Pos Board B311

Experimental Apparatus for Simultaneous Trapping And Nanometer-precision Localization of Single Biomolecules

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The development and continuous improvement of single molecule techniques have elucidated the mechanics of numerous ubiquitous subcellular processes step-by-step, previously inaccessible by conventional average-based biochemical studies. At present, particularly fruitful is the combination of different single molecule techniques in the same setup. In this work we have developed an experimental apparatus which allows the simultaneous detection of the position of a single processive biomolecule, together with mechanical control of its

track/substrate: we have combined single molecule nanometer-precision fluorescence imaging and trapping (FIAT). The performance of this apparatus was tested on a DNA molecule labeled with a single quantum dot and assembled within a microfluidic laminar flow cell between double optically trapped microspheres, in a suspended ''dumbbell'' configuration. In general this assay allows the control of the mechanical conditions of a biological track (actin, microtubules, nucleic acids), while simultaneously monitoring, by fluorescence, translocation (and, possibly, biochemical state) of a molecular motor on the track, without any requirement of surface immobilization. Accordingly, we are now interested on the application of these techniques in the study of transcriptional and translational apparatus, still not fully elucidated at the single-molecule level.

1468-Pos Board B312

Measurement Of The Non-conservative Force Generated By Optical Tweezers

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Optical tweezers have been widely used by biophysicists to measure forces in molecular processes on the single molecule level, such as the force generated by a motor molecular or the force required to unfold RNA. In these and similar force measurements, the usual assumption is that the force applied to a particle inside the tweezers is proportional to the displacement of the particle away from the trapping center, which would imply that the force field is conservative. However, the Gaussian beam model has indicated that the force field generated by optical tweezers is actually non-conservative, yet no experiments have measured or accounted for this effect. We introduce an experimental method that can measure the force field in optical tweezers with high precision without any assumptions about the functional form of the force field. The force field is determined by analyzing the Brownian motion of a trapped particle. We successfully measure the 3D force field with 10 nm resolution for a particle in the Rayleigh regime. The results can be well-approximated with the Gaussian beam model for small displacements, and the non-conservative effect becomes more prominent as the trapped particle is pulled farther away from the trapping center. The energy put into the system along different paths can be directly calculated using the force field. The assumption that Hooke's law applies to optical tweezers neglects the non-conservative component of the force field and can introduce a systematic error when measuring the force.

1469-Pos Board B313

An Optical Torque Wrench For Studying Kinesin Dynamics

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We constructed an optical tweezers instrument capable of exerting torque and measuring the angular motions of small trapped particles, based on the rotation of linear polarization of the trapping laser beam. To change polarization, we employed an electro-optic modulator (EOM), which allows for a much simpler setup than a previous design (La Porta, A. and Wang, M.D. 2004. Optical torque wrench: angular trapping, rotation, and torque detection of quartz microparticles. *Phys. Rev. Lett.* 19:190801). Torque is monitored by measuring the difference between circularly left-handed and right-handed components of the transmitted beam: constant torque is implemented by feeding this angular signal back into a custom-designed electronic servo loop. The limited dynamic range of the EOM ($\pm 180^\circ$) is extended by monitoring the drive signal with a microcontroller, which triggers a switch to flip the output polarization by $\pm 180^\circ$ once a pre-set threshold is reached (within 10 μs). These features enable us to maintain constant torque over unlimited rotations at high bandwidth (~100 kHz). In addition, we developed optically birefringent, non-spherical particles suitable for this instrument using nanofabrication techniques. The polarization-sensitive method employed by the apparatus precludes the use of Wollaston prisms to perform differential interference contrast (DIC) imaging. However, by exploiting conventional video-enhancement techniques (including background subtraction, contrast enhancement, and frame averaging), we report that individual microtubules (~25 nm in diameter) can be visualized without DIC optics at ~5 frames per second. Altogether, our instrument allows for the simultaneous application of force and torque to the study of macromolecules of interest. We are presently extending our previous studies (Gutierrez-Medina, B., et al. 2339-Pos. Torsional properties of kinesin. *Biophys. J.* 2008. 94:2339-Pos) on the torsional properties of the molecular motor kinesin to investigate the effect of torque on its stepwise motion.

1470-Pos Board B314

A Novel Method For Investigating The Azimuthal Rotation Of Molecular Motors Utilizing Dielectrophoresis And Optical Tweezers

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Molecular motors are studied *in vitro* to understand their Biophysics and Cell Biology. Most mechanical studies of myosin and other molecular motors have utilized surface-immobilized motors or filaments, which impact their range of motion. One way of avoiding surface immobilization of the filament and motor is to suspend filaments from fixed supports, giving the motor or the motor-coated cargo unimpeded freedom of motion about its track (Ali et al., *Nat Struct Biol.* 9:464, 2002). We used dielectrophoresis at 4-12 V, 2 MHz to stretch and suspend actin filaments across a 2 x 7 um^2 trench etched between two gold electrodes patterned on a glass slide. Optical tweezers were used to bring a myosin-coated bead into close proximity to a pre-selected, suspended actin filament, facilitating bead attachment to the filament in motility buffer. Using defocused images, the bead's three-dimensional position was tracked as a function of time to obtain its trajectory on the actin. Experiments were carried out with myosin V and myosin X. Both motor proteins followed left-handed helical paths with 1.5 - 2 um pitch. Variants of this technique will enable types of higher complexity found in cells to be addressed with *in vitro* experiments. We thank Drs. Mitsuo Ikebe and Osamu Sato for the gift of myosin X and the Nano/Bio Interface Center (NSF NSEC DMR-0425780) and the NIH (grant AR26846) for support.

1471-Pos Board B315

A High-Resolution Magnetic Tweezer for the Single-Molecule Study of DNA-Protein Interactions

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The magnetic tweezer is a powerful, simple tool to stretch single DNA molecules that tether a magnetic bead to a glass surface. The response of a tethered DNA molecule to interactions with proteins is measured by optical tracking of the bead. Traditionally, bead position is measured by analyzing the bead's diffraction pattern when viewed in a transmitted-light geometry. This approach is straightforward to use, and gives an intrinsic resolution (i.e. ignoring the bead's thermal fluctuations) in measured DNA length of ~ 2nm at 60Hz [1]. This resolution is acceptable when the bead's thermal fluctuations are large, but it disallows measurement of sub-nanometer DNA length changes in the high-force regime, where the fluctuations are reduced below the intrinsic resolution. To obtain sub-nanometer resolution in a magnetic tweezer, we have adapted Reflection Interference Contrast Microscopy (RICM), in which objective-side illumination creates an interference pattern between rays reflecting from the glass and bead surfaces: the interference fringes from RICM vary with bead height nearly twenty-fold faster than diffraction fringes. We have improved the intensity and contrast of the RICM interferogram by fabricating thin films on the bead and glass that optimize the optical properties of those surfaces. We have also removed effects of thermal drift from the system by implementing feedback control of the focal position through piezo-driven motion of the objective [2]. Using the RICM-based method, the intrinsic resolution is improved to 0.12nm at 60Hz, and is stable over one hour. We demonstrate the correct calibration of this method using the force-induced unfolding of DNA hairpins, and we present preliminary data on RICM-measured DNA-protein interactions.

1. N. Ribeck & O.A. Saleh, *Rev. Sci. Instrum.* **79**, 094301 (2008).
2. K. Kim & O.A. Saleh, *Appl. Opt.* **47**, 2070 (2008).

1472-Pos Board B316

Viscous Drag Torque on a Rotating Nanofabricated Cylinder Near an Infinite Plane Boundary

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Microrheological and single molecule biological measurements often involve the use of a microscopic probe particle near a surface. On such systems a precise understanding of the hydrodynamic interactions between the particle and the surface is required. Recently nanofabricated nearly cylindrical quartz particles have been used as ideal trapping particles for an angular optical trap. Here the rotational viscous drag torque imposed on a nanofabricated quartz cylinder near a surface is measured with the angular optical trap. The deviation of torque from the Stokes drag relation in solution is measured as a function of distance from the plane surface of a microscope cover glass. The surface effect is found to be significant for distances on the order of the characteristic dimension of the cylinder. These findings will allow for more accurate and quantitative torsional measurements of angular trapping systems.