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.

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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.

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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.

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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