Historically, creating such DNA substrates is inefficient. More problematic is that data throughput is low in standard surface-based assays since all substrates are unwound upon introduction of ATP. The net result is ~2–4 high-resolution traces on a good day. To improve throughput, we sought to turn-on or activate a substrate for a helicase one molecule at a time and thereby sequentially study many molecules on an individual microscope slide. As a first step towards this goal, we engineered a dsDNA that contains two site-specific nicks along the same strand of the dsDNA but no ssDNA. Upon overstretching the DNA (F = 65 pN), the strand between the two nicks was mechanically dissociated. We demonstrated this with two different substrates: one yielding an internal ssDNA region of 1100 nt and the other yielding a 20-bp long hairpin flanked by 30 nt of ssDNA. Unwinding a hairpin yields a 3-fold larger signal while the 30-nt ssDNA serves as the binding site for the helicase. We expect that these force-activated substrates to significantly accelerate high-resolution optical-trapping studies of DNA helicases.

9922-34, Session 6

Highly birefringent TiO2 nanocylinders: characterization and application in the optical torque wrench

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Recent advances in optical tweezers have enabled the direct manipulation and measurement of optical torque using light spin momentum transferred to trapped birefringent particles. This powerful technique, termed Optical Torque Wrench (OTW), relies on trapping of quartz (SiO2) microcylinders which have become a standard and convenient probe for single-molecule studies. Here, we explore an alternative photonic probe based on rutile (TiO2) which has almost thirty-fold larger birefringence compared to quartz particles. By employing this promising material to fabricate rutile nanocylinders whose sizes can be easily tuned, we significantly enhance the accessible range of optical torques and angular frequencies in the OTW. In future, these novel photonic probes will allow us to study not only slowly processing torque-generating biological systems, as the genome processing machinery, but also fast rotating motors, including ATP-synthase and the bacterial flagellar motor.

9922-35, Session 6

Investigation of subcellular localization and dynamics of membrane proteins in living bacteria by combining optical micromanipulation and high-resolution microscopy

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The plasma membrane serves as protective interface between cells and their environment. It also constitutes a hub for selective nutrient uptake and signal transduction. Increasing evidence over the last years indicates that, similar to eukaryotic cells, lateral membrane organization plays an important role in the regulation of prokaryotic signaling pathways. However, the mechanisms underlying this phenomenon are still poorly understood. Spatiotemporal characterization of bacterial signal transduction demands very sensitive high-resolution microscopy techniques due to the low expression levels of most signaling proteins and the small size of bacterial cells. In addition, direct study of subcellular confinement and dynamics of bacterial signaling proteins during the different stages of the signal transduction also requires immobilization in order to avoid cell displacement caused by Brownian motion, local fluid flows and bacterial self-propulsion.

In this work we present a novel approach based on the combination of high resolution imaging and optical manipulation that enables the investigation of the distribution and dynamics of proteins at the bacterial plasma membrane. For this purpose, we combine the versatility of holographic optical tweezers (HOT) with the sensitivity and resolution of total internal reflection fluorescence (TIRF) microscopy. Furthermore, we discuss the implementation of microfluidic devices in our integrated HOT+TIRF system for the control of growth conditions of bacterial cells. The capabilities of our workstation provides thus new valuable insights into the fundamental cellular and physical mechanisms underlying the regulation of bacterial signal transduction.

9922-36, Session 6

Thermophoretic trapping and manipulation of single molecules

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The strength Brownian fluctuations of objects in solution increase with increasing temperature. Here, we present a method which is capable of trapping single and multiple molecules in solution by spatially and temporally varying temperature fields. The highly localized temperature fields are generated by an optical heating of a plasmonic nanostructure. The resulting temperature gradients induce thermodiffusive drift of the trapped object away from the heat source. A feedback controlled switching of the repulsive temperature gradient is introduced, which allows us to confine the motion of a single DNA molecule for several minutes. The feedback rules allow for a precise control of the effective trapping potential shape. Due to the inhomogeneous character of the temperature field, the trapping of a well defined number of single molecules is possible, which paves the way for new molecular interactions studies.

9922-37, Session 6

Temperature control and measurement with tunable femtosecond optical tweezers

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We present effects of wavelength dependent temperature rise in a femtosecond pulsed optical tweezer. Our experiments involve trapping a 1-micron polystyrene bead in a dilute aqueous IR dye solution with a low power (25 mW) femtosecond laser that is tunable in the NIR range (730 820 nm). The tunable NIR trapping laser can resonantly excite the homogeneous solution of IR-dye resulting in a heated trapped volume. The high repetition rate laser acts as a resonant excitation source to create local heating effortlessly within the trapping volume. We have used both equipartion theorem and power spectrum method to evaluate temperature at different wavelengths having different absorption coefficients. Fixed pulse width in temporal domain corresponds to a constant spectral bandwidth, which makes our setup a high precision tunable temperature rise controller. This observation leads us to calculate temperature as well as viscosity within the vicinity of the trapping zone. A mutual energy transfer occurs between the trapped bead and the surrounding solution that leads to the transfer of thermal energy of the dye into the kinetic energy of the trapped bead and vice-versa. Thus hot solvated dyes resulting from resonant and near resonant excitation around trapping volume can continuously dissipate heat to the trapped bead, which will be reflected in the frequency spectrum of Brownian noise exhibited by the trapped bead. Temperature rise near the trapping zone can significantly change viscosity of the medium. We observe temperature rise profile according to its Gaussian absorption spectrum at different wavelengths.