[3] H. Shimizu et al., Cell 132:67-78 (2008)
[4] T. Kanzaki et al., J. Biol. Chem. 283:34773-34784

973-Pos

Optimal Estimation of the Diffusion Coefficient from Noisy Time-Series Measurements

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Single-molecule time-lapse measurements of diffusing proteins often contain considerable localization error. The standard method for estimating the diffusion coefficient is based on the mean square displacements. This method is highly inefficient, since it ignores the high correlations inherent to these. A Generalized least squares method, which takes into account these correlations, is presented and it is shown that it attains the maximum precision possible according to information theory. The method is demonstrated on data from high-speed time-lapse photography of the hOgg1 repair protein diffusing on DNA.

974-Pos

Adaptive Platform for Highly Parallel Low-Noise Recordings of Single Membrane Proteins

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Highly parallel, low noise electrophysiological recordings of single ion channels are of interest both for basic research and drug development. Here, a microsystems approach is presented (see Fig.1A) which greatly simplifies the recording configuration and optimizes the electrical parameters governing noise. The lipid bilayer is formed on a picoliter cavity generated within a photochemical resist acting as a dielectric (see Fig. 1B). On the bottom of each cavity a microelectrode is placed.

Using standard photolithographical techniques this design allows for many such setups on one chip, and is therefore in principle well suited for highly parallel single channel recordings. Parallel recordings (16-electrode multiarray) of currents mediated by alamethicin are shown (see Fig.1C), illustrating the potential of this novel approach towards high-throughput measurements of single membrane proteins. Furthermore, the flexibility of this approach is outlined by the reconstitution of α-hemolysin in the lipid bilayer. The capability for molecular analysis is demonstrated by the detection of oligomers diffusing through the bacterial pores.

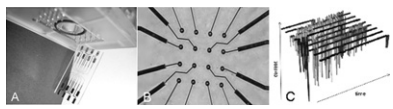

Fig. 1. *Patch16Chip* ***(B)*** *Connecting lines and cavities over the circular shaped electrodes.* ***(C)*** *Current traces of single alamethicin-channels, detected in parallel at 9 different cavities.*

975-Pos

A New Closed Cell, Horizontal Magnetic Tweezer

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We report on the development of a magnetic force transducer or tweezer that can apply piconewton forces on single DNA molecules in the focus or horizontal plane. Since changes in the DNA's end-to-end extension are coplanar with the pulling force there is no requirement to continually refocus the tethered beads thus considerably simplifying single molecule micromanipulation experiments in our setup. The DNA constructs (γ-DNA end-labeled with a 3μm polystyrene bead and a 2.8μm magnetic sphere) and buffer are introduced into a 200μL to 500 μL closed cell created by using two glass slides separated by 1mm spacers and a thin viscoelastic perimeter wall. This closed cell configuration isolates our sample and produces low-noise force and extension measurements. Breaching the viscoelastic barrier are five pipettes: a 1-2μm inner diameter suction pipette used for capturing the polystyrene bead, a magnet-tipped pipette used to pull the magnetic sphere, a 0.5mm-inner-diameter injection pipette used to introduce proteins of interest, and two 0.5mm-inner-diameter pipettes used to maintain flow. The suction micropipette and the injection pipette are rigidly coupled and positioned by a manual three axis manipulator that can produce continuous displacements of 15mm, 25mm and 25mm in the x-, y-, and z-axis respectively. The magnet-tipped pipette is controlled by a motorized two axis micromanipulator capable of continuously spanning the full width of the cell. A motorized micromanipulator with a defined 157nm step size maneuvers the cell over a 32X/0.40NA objective and between the two mechanical manipulators. Initial tests show the capability of the device to easily and repeatedly find, capture, and manipulate end-labeled DNA constructs.

976-Pos

Optical Trapping and Two-Photon Exitations of Quantum Dots

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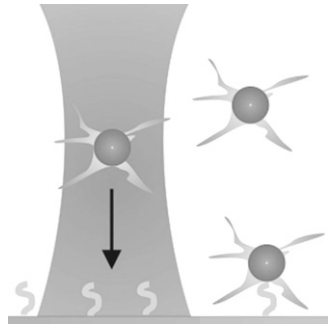
Individual colloidal quantum dots can be optically trapped and manipulated by a single infrared laserbeam operated a low laser powers [Jauffred et al. Nano letters 2008 (**10**)] and the spring constant of the force, exerted by the harmonic optical trap on a quantum dot, have been found to be of the order of 10^{-4} pN/nm. We measured the optical trapping strength of individual colloidal quantum dots with different emission wavelenghts (from 525 nm to 800 nm) and different physical sizes, with the result that these diverse quantum dots have identical trapping capabilities.



Furthermore, we show that the trapping laserlight can also act as a source for two-photon excitation of the trapped quantum dots, thus eliminating the demand for an excitation light source in addition to the trapping laser beam.

977-Pos

Optical Torque Wrench for Single Molecule Studies

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At the molecular level, the torque applied to biopolymers plays a central role in many processes involving their conformational changes and interactions with proteins. We will study the torque-sensitivity of individual nucleic acid molecules and their interactions with proteins using a novel optical tweezers configuration termed the optical torque wrench [1].

In standard single-molecule techniques, torque cannot be simultaneously controlled and detected, in contrast with the case of the applied force. With this new technique we will be able to control both parameters in real-time in single molecules of DNA or RNA, with high temporal (250 kHz) and spatial (nm) resolution typical of optical tweezers. Here we present a first characterization of our instrument.

This will allow us to acquire fundamental insight into the torque-sensitivity and dynamics of nucleic acids, DNA packaging, polymerase activity in DNA replication or transcription, and related biological processes.

[1] A. La Porta and M.D.Wang, Phys. Rev. Lett. 92, 190801, 2004.

Atomic Force Spectroscopy

978-Pos

Fishing on Living Cells with AFM: Novel Method to Study Topology and Dynamics of Cotransporter SGLT1 Protein

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In the apical membrane of epithelial cells from the small intestine and the kidney, the high-affinity Na^+/D-glucose cotransporter SGLT1 plays a crucial role in intestinal glucose absorption and in renal glucose reabsorption. Here the over-expression of rabbit SGLT1 in rbSGLT1-transfected Chinese hamster ovary (CHO) cells was first characterized using the immuno-staining method on non-permeabilized cells. The functionality of the SGLT1 was verified by biochemical approaches. Atomic force microscopy (AFM) was employed to probe initial substrate-carrier interactions, topology, and function of SGLT1 in living cells on the single-molecule level. Specific recognition events in force distance cycles were detected using epitope specific antibodies or thio-glucoside bound to the AFM tip. Upon addition of D-glucose or the specific inhibitor phlorizin, binding of antibody primed AFM tips drastically decreased suggesting recognition sites for D-glucose in the extracellular loop 8-9 and for phlorizin in the extracellular loop 13-14. The binding probability of the thio-D-Glucose tip was reduced by various sugars in a potency sequence that differed markedly from transport studies. We therefore propose that the first of several selectivity filters of SGLT1 is formed by the two extracellular loops 8-9 and