1010-Pos

Modeling of DNA in Nanochannels using Linear Elasticity Theory

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We model the dynamics of a single, fluorescence-dyed DNA molecule in a nanochannel. When a single dsDNA molecule is placed in a nanochannel, its extension along the channel is up to 50% of its contour length, depending on the dimensions of the channel. This linear extension offers the possibility to study, e.g., the binding of site-specific proteins or the sequence-dependent melting of DNA, with the latter giving a coarse-grained representation of the sequence [W. Reisner *et al*., ''Single Molecule Denaturation Mapping of DNA in Nanofluidic Channels.'' Resubmitted to *Nature Nanotechnology* (2009)]. The resolution achieved with this approach has two limiting factors: The diffraction limit, which for isolated probes can be circumvented with FIONA, and thermal density fluctuations of the DNA in the channel. Density fluctuations make distances measured along the channel correspond to distances measured along the molecule in an unknown non-linear and random, time-dependent manner.
This last problem can be circumvented if the non-linear correspondence between coordinates can be extracted from images at any point in time. To this end, we model the thermal motion of the molecule using linear elasticity theory combined with Boltzmann statistics. The thermal dynamics of the resulting model is consistent with movies of DNA displaying thermal density fluctuations.

1011-Pos

Ultrathin Nanoporous Silicon Nitride Membranes for Separations and Biosensing

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Separation processes and biosensing are interesting applications for single- and multiple-nanopore membranes. Pore density, surface chemistry and shape are determining factors of the membrane capabilities in these applications. We present ultrathin freestanding SiN membranes with conical or double-conical nanopores prepared by track-etching. This technique allows the membrane porosity to be tuned from a single pore to billions of pores per cm^2, and these pores have diameters as small as several nanometers. These conical and double-conical pores give higher permeant fluxes compared to standard membranes with cylindrical pores, which is a key advantage in separation applications. To show the separation capabilities of these membranes, we discriminate between dye and protein molecules based on their size and charge. This separation process operates in physiological electrolyte conditions and is based on an electrostatic mechanism. We also demonstrate that by chemically modifying the pore surface, we can tune the separation behavior. In addition, the conical pore shape results in a shorter effective length region which dominates the pore's electrical resistance. This is advantageous for single biomolecule detection applications such as nanopore-based DNA analysis because the detected signal corresponds to a smaller region of the analyte molecule. We present initial results with single-pore membranes evaluating these advantages.

1012-Pos

Electrochemical Detection of Signalling Responses in Excitatory and Non Excitatory Cells using Chemoreceptive Neuron MOS Transistors(CVMOS)

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Transistor based techniques show tremendous potential to detect cellular events with high temporal resolution at the single cell level. We report on a label-free electronic technique using Chemoreceptive MOS transistors (CVMOS) to study the response to stimulation of excitable and non excitable cells. CVMOS charge sensors provide independent gate bias control facilitating capacitive amplification and reference electrode less operation. As a proof of concept, we use this CMOS platform to detect the response of RBL-2H3 mast cells to stimulation mediated through IgE and its high affinity cell surface receptor, FcϵRI, using the antigen DNP-BSA on a population of cells. I -V characteristics of the transistor and constant voltage recordings at high temporal resolution suggest changes of extracellular charge and/or capacitance upon stimulation. We observe a shift in the drain current as stimulation is initiated, followed later by current fluctuations that show a time course similar to those of amperometric recordings. The responses are dependent on the presence of extracellular calcium, suggesting that the observed changes may be linked to exocytosis. Unsensitized cells show no detectable response to antigen stimulation. Using adrenal chromaffin cells, we observed rapid current fluctuations in response to stimulation with both ionomycin and high KCl. Experiments are underway to determine whether these responses reflect stimulation action potentials and/or catecholamine release events.

1013-Pos

Geometric Sensing in Cells - a Molecular Approach

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Cells sense changes in substrate geometry and respond by modifying their behavior. Finding the dimensions and characteristics that elicit a response is crucial to understand this phenomenon. To analyze this, grooves and ridges with width = 1.2 μm, pitch = 3μm and varying heights (1μm to 50 nm) were fabricated in fused silica using photolithography and dry etching. Fused silica was used as material as the roughness post etching is very little (less than 8 nm). Also, to probe the effect of curvature on the cells, the grooves and ridges were given a curvature using shallow trench fabrication process. In this method the grooves and ridges are made using photolithography and dry etching, which is followed by controlled oxide deposition leading to curved sidewalls. Fibroblasts were seeded and fixed after 75 min of spreading. DIC and actin immunofluorescence was used to image the cells. Plots of spreading area and anisotropy ratio v/s different heights revealed that a height of 100 nm is the minimum feature height that the cells can detect. Below this height, they tend to spread isotropically as on a plane glass coverslip. Above 100 nm the cells tend to align in the direction of the grooves and show strong alignment with increased heights of ridges. Currently we are looking at the effect of curvature on the cells.

1014-Pos

Use of Arrays of Releasable Microstructures for Selection of Single Cells and Colonies

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Microfabricated arrays of individually releasable elements have been developed to enable cell analysis and isolation of cells from small cell samples, typically less than 4,000 cells. A cell suspension is added to the array and the cells are captured as the settle onto the individual elements. After capture, cells are cultured for the desired length of time. By plating single-cell suspensions of fewer cells than elements, one or fewer cells are captured per element. Prolonged culture then enables growth of clonal colonies on the array. Standard microscopic imaging is used to identify cells or colonies of interest, and individual elements containing target cells are then released and collected. Creation of arrays composed of elements with various geometries and three dimensional structures has been accomplished to maximize capture efficiency of cells plated on the array and growth of clonal colonies. This work has led to the ability to pattern both adherent and nonadherent cells, with subsequent selection and collection of single cells or clonal colonies with unique characteristics. Surface modification of the elements has been accomplished to enrich the cell samples for particular cell types. Selective release of the individual elements along with the resident cells has been enhanced to achieve high collection efficiencies of viable cells which can then be expanded under standard tissue culture conditions. Three dimensional elements have also been created that enable cell growth in isolated cell culture microenvironments which will prove valuable for cell-cell interaction studies.

1015-Pos

A Platform for Supramolecular Nanochemistry

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There is increased need for analytical tools to probe (bio)molecular recognition and chemical interactions. This can be provided by nanochemistry, where the focus is on individual molecules, or molecular assemblies that, unlike bulk canonical ensembles, exhibit some degree of order. Nanochemical systems rely on self-assembly and (bio)molecular recognition, and one way to introduce order into a system is to link these processes to surfaces. We here present a 2D micro-/nano-fluidic technique where reactant-doped liquid crystal (LC) films spread and mix on micropatterned amphiphilic substrates. These phospholipid monolayer films are individually doped with complementary DNA strands modified in one end by a lipophilic anchor and with a fluorescent dye in the other end. By using fluorescence resonance energy transfer we monitor the hybridization of the complementary strands. Our results show that the density of reactants, number of different reactants as well as the sequence of reactant addition, can be controlled within LC films confined to micromachined substrates. The technology introduced here provides a platform for nanochemistry with potential for kinetic control where molecular assemblies with 2D orientational order can be established, controlled, and probed.