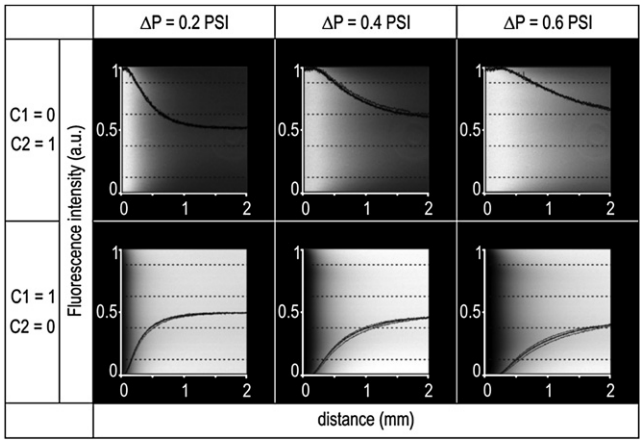
effects near walls <25% of area). Due to the binary branching of the inlets, the perfused area can be arbitrarily wide (here, 2 mm). This enables analyzing responses of large cell populations in parallel and acquiring rich statistics on single-cell variability.



255-Pos Board B134

Detection and Identification of Virus Particles on a Microfluidic Platform

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We have developed a nanoparticle-based assay to capture and detect targeted virus particles on a microfluidic platform. Antibody-decorated magnetic nanoparticles were used to capture virions and detection was afforded by measuring the change in magnetic birefringence relaxation rates. Vesicular Stomatitis Virus (VSV), a bullet-shaped, negative-strand RNA virus, is used as a model for air, water or blood-borne pathogenic viruses. Antibodies against the single envelope glycoprotein of either the Indiana or New Jersey strains of VSV were conjugated to 30nm superparamagnetic nanoparticles to generate the capturing reagent. The size distribution of the antibody-nanoparticle conjugates was determined using a CONTIN analysis of dynamic light scattering (DLS) data. Our results were confirmed by TEM analysis. Antibody-decorated nanoparticles were combined with one of the VSV strains and subjected to Ismagilov mixing, utilizing two immiscible phases in micro-channels to increase the speed of capture. Virus binding was determined by changes in hydrodynamic volume of the virus-nanoparticle complexes as measured by their birefringence relaxation rate under stopped flow conditions. In addition, the dynamics of particle complex formation and aggregation was studied by DLS at different scattering angles. We compared the results of these two methods for measuring time-dependent increases in the size of the nanoparticle/virus complexes. The birefringence relaxation method is more adaptable to field and other applications than DLS since it is relatively insensitive to particle concentration or to the presence of dust and sample impurities. Our approach is superior to existing techniques such as qPCR or ELISA assays due to the speed of detection and insensitivity to environmental contaminants. We are looking into the suitability of this assay for point-of-care applications.

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Phenotypic and Genotypic Heterogeneity of Cyanobacterial Populations in Hot Spring Microbial Mats Revealed by Microfluidic Single-Cell Analysis

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The microbial mats found at Octopus Spring in Yellowstone National Park exhibit highly organized community structures. Thermophilic unicellular cyanobacteria (Synechococcus spp.), found in the 1-mm thick top layer of these hot spring mats, act as the primary producers in the microbial communities. The cyanobacterial population is an excellent model system for studying how environmental factors affect the structure of a microbial community because the system is relatively simple and formed under a well-defined set of environmental gradients such as temperature, oxygen and light levels. To obtain a detailed description of both phenotypic and genotypic structures of the population, we extended our microfluidic approach that has been previously developed for single-cell protein analysis of a similar type of cells [Huang et al., Science, 315, 81-84 (2007)]. First, a protein analysis chip was developed for simultaneous analysis of multiple single-cell lysates for higher throughput. We demonstrate that 16 cells can be analyzed individually during each round of analytical procedures to obtain phycobiliprotein distributions at the single-cell level. Second, a genetic analysis chip was designed to amplify genomic DNA from individual cyanobacteria cells via multiple displacement amplification. The presence of a selected set of genes was compared among populations under different environmental conditions. This type of single-cell genomic data is useful for elucidating the role of cyanobacterial species deduced from the metagenomic analysis of the microbial mat samples.

257-Pos Board B136

Adsorption and Stability of Streptavidin on Cluster-Assembled Nanostructured TiO*x* Films

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The study of the adsorption of proteins on nanostructured surfaces is of fundamental importance to understand and control cell-surface interactions and, notably, cell adhesion and proliferation; it can also play a strategic role in the design and fabrication of nanostructured devices for postgenomic and proteomic applications. We have recently demonstrated that cluster-assembled nanostructured TiO*x* films produced by supersonic cluster beam deposition [1] possess excellent biocompatibility and that these films can be functionalized with streptavidin, allowing the immobilization of biotinylated retroviral particles and the realization of living-cell microarrays for phenotype screening [2,3].
Here we present a multitechnique investigation of the adsorption mechanisms of streptavidin on cluster-assembled TiO*x* films. We show that this nanostructured surface provides an optimal balance between adsorption efficacy and protein functionality. By using low-resolution protein arrays, we demonstrate that a layer of adsorbed streptavidin can be stably maintained on a cluster-assembled TiO*x* surface under cell culture conditions and that streptavidin retains its biological activity in the adsorbed layer. The adsorption mechanisms are investigated by atomic force microscopy in force spectroscopy mode and by valence-band photoemission spectroscopy, highlighting the potential role of the interaction of the exposed carboxyl groups on streptavidin with the titanium atoms of the nanostructured surface.

[1] Barborini E. et al., J Phys D: Appl Phys 1999;32:L105-9.
[2] Carbone R. et al., Biomaterials 2006;27(17):3221(9).
[3] Carbone R. et al., Biomaterials 2007;28(13):2244(53).

258-Pos Board B137

A "Microfluidic Nose": Detection of Olfactory Sensory Neuron Responses to Odorants Across the Whole Olfactory Receptor Space

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In vertebrates, odorant molecules are detected by olfactory sensory neurons (OSNs) present in the nasal epithelium. A large, G-protein coupled receptor gene family is responsible for transducing the detection of a cognate molecule for a given receptor. Each OSN appears to express only one of thousands of olfactory receptor (OR) genes in rodents. Odorants are perceived by combinatorial activation of a number of ORs (it is specific to a subset of odorants); each OR recognizes a range of odorants and odorants are typically recognized by a number of ORs. Given the approximately thousand OSN/OR types and the hundreds of thousands of potential ligands, measuring individual OSN activation with the usual *in-vivo* and *in-vitro* methods is a laborious task that is not suitable for interrogating the whole OR space. Hence a microfluidic and high-throughput system was developed to analyze these cells.
Utilizing the techniques of soft-lithography, we developed a microwell array of ~32,000 wells (20 um diameter, 10 um depth) to capture dissociated olfactory epithelia (OE) cells and sequentially exposing them to different odorants. Cell response was detected using the Fluo4AM calcium binding dye. By imaging the fluorescence change in each well, a response profile to each odorant can be constructed for thousands of individual OSNs simultaneously.

259-Pos Board B138

SOI Nanofet Devices For Ultra-Sensitive Detection of Biomolecules

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In this work the fabrication and fluidic testing of silicon-on-insulator (SOI) field effect devices for the label-free detection of biological molecules are presented.

Devices with widths varying from the nanoscale (50 nm) to the microscale (2 um) regime are fabricated on the same chip using standard top-down microfabrication techniques and electron beam lithography. During the fabrication, several steps focus on ensuring that the resulting devices have proper insulation for stable operation in fluidic environments, including a deposited high integrity passivation layer to insulate all but the active areas of the devices. Both normal dry and wet electronic operation of the FET devices are demonstrated. The resulting devices are observed to be fully functional in ionic fluid environments up to very high voltages, with much longer lifetimes than other comparable devices in the literature. Further, the devices are shown to be highly sensitive to charge, using both DC and AC sensing schemes.

Though much work has been completed over the past few years on similar devices, such important factors as device characteristics, sensing schemes, and testing parameters vary widely from work to work. It not yet clear what factors need to be optimized towards the goal of realizing maximal device sensitivity, or how these factors should be optimized. An experimental analysis is presented towards such an optimization, including a detailed characterization of the effect of device width on the final device sensitivity (enabled by the simultaneous fabrication of both nanowire and ''nanoplate'' devices on the same platform), a comparison of AC and DC sensing schemes, and the optimization of operating biases while sensing. This analysis enables further work to proceed in the most efficient and informative way possible to reach the maximum sensitivity limit of the devices to target analytes.

260-Pos Board B139

Molecular Scale Dielectric Sensors for Highly Sensitive Biomolecular Detection

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Capacitive sensors provide a promising alternative to conventional optical methods of detecting biomolecular interactions, due to their label-free operation, simple instrumentation and ease of miniaturization. Although several configurations of capacitive biosensors have been reported in the literature, physical and electrochemical properties of these structures and the measurement methods used have significantly limited their commercial development as biosensors. The existence of electrode polarization effect and noise from solution conductance limited the earlier dielectric spectroscopic measurements to high frequencies only, which in turn limited their sensitivity to biomolecular interactions, as the applied excitation signals were too fast for the charged macromolecules to respond. The series parasitic impedance from electrode polarization effect masked the dielectric changes occurring due to biomolecular interactions at low frequencies (<1 kHz).

To address such challenges, we have developed a molecular scale capacitive sensing device with an electrode separation < 30nm. This nano-scale sensing area provides a window into bio-molecular interactions which was not previously attainable with macro or even micro scale devices. The interaction between the electrical double layers due to the space confinement decreases the potential drop across the electrode spacing and allows dielectric measurements at low frequency. As the double layers from both the capacitive electrodes merge together and occupy a major fraction of the dielectric volume, the contribution from bulk sample resistance in the measured impedance is eliminated. The dielectric properties during nucleic acid-protein interactions were measured using alpha thrombin and its aptamer. A 45-50% change in capacitance was observed due to aptamer-alpha thrombin binding at 10Hz. Highly sensitive capacitive detection of nucleic acid hybridization reactions was also demonstrated.

261-Pos Board B140

Miniaturized Ion Channel Reconstitution Platform Based On Silicon Microfabrication

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Currently, ion channel reconstitution is performed into lipid bilayer membranes which are suspended across apertures of a diameter of 100 μm - 250 μm. When compared with the size of membrane patches in patch-clamp experiments, these suspended membranes have a significantly larger area (up to a factor of 15000), which makes them more fragile and increases the source capacitance. Latter introduces feedback noise, which limits the signal-to-noise ratio of the measurement setup. We have fabricated apertures in a silicon substrate with diameters between 5 μm and 50 μm across which lipid bilayers can be formed. The aperture has been tapered to allow for the thinning of the membrane, similar to the established thin plastic sheets. The surface of the silicon chip has been hydrophobically coated to facilitate lipid bilayer formation. Additional SU-8 epoxy coating provides a layer reducing the capacitance of the solid support. This device can act as a direct replacement for the current plastic supports without any change to the lipid bilayer formation protocol.

Results on the lipid bilayer formation probability will be reported along with values on the seal resistance and the capacitance of the setup. Bilayer seal resistance values of up to 60 Gigaohm have been observed repeatedly on these substrates. Spectrally resolved measurements on the noise originating from the lipid bilayers formed across the apertures in silicon will be presented. The setup will be used to demonstrate ion channel reconstitution into the lipid bilayers, using the voltage-gated OmpF ion channel of E. coli as a test for physiological gating activity.

262-Pos Board B141

Rapid Incorporation of Heterologously Expressed GPCR CCR5 in Nanoscale Apolipoprotein Bound Bilayers (NABBs)

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To study reconstituted membrane proteins in a native-like bilayer environment outside of the cell, we developed a novel self-assembling system using zebrafish apolipoprotein A-I (zap1). Nanoscale apolipoprotein bound bilayers (NABBs) are stable discoidal structures that allow access to both topological surfaces of transmembrane receptors. We showed earlier that the prototypical G protein-coupled receptor (GPCR), rhodopsin, is stable and functional when reconstituted into NABBs. [1] Here we report the incorporation into NABBs of an engineered C-C chemokine receptor 5 (CCR5) - a rhodopsin-like GPCR involved in the immune response and used as the primary coreceptor for HIV-1. Recombinant CCR5 was immunoaffinity purified from detergent extracts of a mammalian cell line. CCR5-NABBs were prepared by mixing phospholipids, zap1 and purified CCR5 followed by hydrophobic affinity chromatography to remove detergent. The resulting crude NABBs were purified by size exclusion chromatography. Using a novel sandwich ELISA method, we quantified the yield of correctly-folded CCR5. The CCR5-NABBs induced nucleotide exchange by heterotrimeric G proteins in response to the agonist chemokine ligand, CCL5 (RANTES). We plan to carry out structural and dynamic studies of the reconstituted ternary complex of agonist, receptor, and heterotrimeric G protein. NABBs appear to be a flexible tool for a variety of biophysical studies of engineered heterologously expressed GPCRs.

[1] S. Banerjee, T. Huber, T.P. Sakmar. 2008. Rapid Incorporation of Functional Rhodopsin into Nanoscale Apolipoprotein Bound Bilayer (NABB) Particles. *J. Mol. Biol.* **377**, 1067-1081.

263-Pos Board B142

Nanotubes As Drug Delivery Systems For Prokaryotic And Eukaryotic Cells

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A major challenge for drug delivery techniques is to overcome the barrier imposed by the cell membrane. In the past this has been addressed by e.g. permeabilization of the membrane with lipids, electric currents, or toxins, and by physical penetration with microprojectiles. A common issue is physical damage to the cell membrane. Nanotechnology offers the possibility of ''nano-injectors/carriers'' that penetrate cell membranes with minimal perturbation. This will require both a more fundamental understanding of how nanoparticles interact with cell membranes and their components, and of how to avoid toxicological side effects via unwanted membrane perturbations. There has been no systematic study of e.g. which parameters determine whether or nor CNTs penetrate membranes, or of the nature of their interaction with different cell organelles.

Here we present a systematic study of the effect of CNT (both SWNTs and multi-walled carbon nanotubes, MWNTs) on living prokaryotic (E. coli) and eukaryotic cells (S. cerevissiae) using AFM, and environmental SEM (ESEM). The influence of CNT length and diameter, surface chemistry (e.g. by introduction of carboxylic groups by oxidation), functionalisation by coating with phospholipid bilayers, proteins or double-stranded DNA on these interactions is studied. Acid oxidation and nanotube doping is used for controlling the length and diameter of MWNTs and for modifying their surface chemistry (Burch, Brown, Contera, et al. J. Phys. Chem. C, 2008). We have demonstrated that specific surface chemistry, and CNT diameter are crucial for achieving the coating of CNTs with correctly folded proteins (Burch, Contera, et al., Nanotechnology, 2008) and phosholipid bilayers (Toledo, de Planque, Contera & Ryan, Jap. J. Appl. Phys. 2007, and JACS submitted). The identification of the CNTs inside cells and of their specific interactions with organelles is done with ESEM.