View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector



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**

**Manu Sebastian Mannoor**[1], Teena James[1], Dentcho V. Ivanov[1], William Braunlin[2], Les Beadling[2].
[1]New Jersey Institute of Technology, Newark, NJ, USA, [2]Rational Affinity Devices, Newark, NJ, USA.

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**

**Michael Goryll**, Nipun Chaplot.
Arizona State University, Tempe, AZ, USA.

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)**

**Sourabh Banerjee**, Amy Grunbeck, Thomas Huber, Pallavi Sachdev, Thomas P. Sakmar.
The Rockefeller University, New York, NY, USA.

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**

**Sonia Antoranz Contera**, Sonia Trigueros, J.F. Ryan.
University of Oxford, Oxford, United Kingdom.

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.