by leak currents through nanopores and respective shifts in the transmembrane ion balance. We employed whole-cell patch-clamp to explore the effect of 300-ns USEP on voltage-gated sodium channels in neuroblastoma cells (NG108). We found that a single USEP could inhibit VG INa , with the threshold at about 1.8 kV/cm. Voltage-dependent activation and inactivation curves shifted to more negative membrane potentials: V0.5 of activation moved from -22.8 ± 0.2mV before USEP to -26.4 ± 0.6mV after it (mean $\pm$ s.e.), and V0.5 of inactivation changed from -65.9 ± 0.2mV to -72.2 ± 0.2, respectively; the slope factor did not change. Concurrently, USEP exposures induced a non-inactivating, voltage-sensitive inward current due to nanopore formation. The presence of 100μM Gd3+ in the bath buffer significantly reduced the nanopore current and also eliminated the inhibitory effect of USEP on VG INa. This finding suggests that USEP-induced inhibition of VG INa. and changes in its kinetic characteristics may be mediated by opening of nanopores and consequent alterations of the ion equilibrium.
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3123-Pos
Rectification of a Modified Nanofluidic Diode Dependent on the pH
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We study the affect of varying pH on the rectification of a nanofluidic diode. The diode is a conically shaped nanopore in PET or Kapton that has a distribution of charge along the surface. The distribution is such that there is a boundary where one side is positively charged and the other side is negatively charged. We also measure nanopores that have neutral and negative charges on either side of the boundary. The charges are modified by a 2 step chemical reaction using EDC/PFP for the 1st step and ethylenediamine or propylamine for the second step. Characterization of the nanopore was done by taking I-V curves from −5V to 5V with buffered solutions of KCl.

3124-Pos
Light-Induced Permeability Changes in Liposomes Containing Photo-Polymerizable Phospholipids
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We have designed a novel class of light-triggerable liposomes prepared from a photo-polymerizable phospholipid $DC_{8,9}PC$ (1,2- bis (tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine) and DPPC (1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine). Exposure to UV radiation (254 nm) for 0-45 minutes (25 ^{0}C) resulted in photo-polymerization of $DC_{8,9}PC$ in these liposomes and the release of an encapsulated fluorescent dye (calcein). Photopolymerization and permeability changes did not occur from UV-triggered Egg PC/$DC_{8,9}PC$ liposomes. We propose that phase separation and packing of polymerizable lipids in the liposome bilayer are major determinants of photo-polymerization resulting in the formation of local defects and/or lipidic pores in the liposome membrane. Differential Scanning Calorimetry show phase transition peaks at 36.8 ^{0}C and 41.6 ^{0}C, respectively, in liposomes composed of DPPC:$DC_{8,9}PC$ (9:1 mole ratio) indicating that the reactions occurred while these lipids are in the gel phase (25 ^{0}C). Our results indicate that $DC_{8,9}PC$ and DPPC molecules undergo de-mixing in the gel phase. This hypothesis is supported by Molecular Dynamics simulations that indicate separation of $DC_{8,9}PC$ and DPPC in the solid phase lipid bilayer. Cryo-electron microscopic images of the liposomes show major changes in liposome morphology after UV irradiation. When an appropriate tunable photo-sensitizer dye is included in the aqueous compartment of these liposomes, release of contents is triggered by excitation with a laser at the wavelength of the encapsulated dye. Inhibition of release in the presence of oxygen radical scavengers indicate that the mechanism of release involves chemical changes in $DC_{8,9}PC$ unrelated to photo-polymerization. The laser-mediated chemical modifications in $DC_{8,9}PC$ are being analyzed by MS, LC, GC and NMR. We are further developing these liposomes for undergo triggered release of chemotherapeutic agents (e.g. doxorubicin) and are testing their efficacy *in vitro* and *in vivo*.

3125-Pos
Label-Free Immunoassay Based on Functionalized Nanopipette Probes
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Nanopipette technology is capable of detecting and functional analyzing biomolecules based on difference on their size, shape and electrical charge. This unique label-free biosensor is inexpensive, easy to fabricate and versatile. It gives a fast and real time output even in small reaction volume (attoliters). At this point, the nanopipette size and geometry, together with the surface chemistry preparation for attachment of a biomarker, antibody or protein probe was optimized by both experiments and modeling to result in detectable signals by the nanopipette. In this phase, the goal of the surface chemistry procedure was to prepare nanopipette tip in a way that only controlled amount of the surface is functionalized and used for probe attachment. Preliminary experiments are demonstrating the sensitivity and selectivity of the technique with specific proteins targeting HPV as well as environmental toxins. These results prove that nanopipettes functionalized with appropriate molecular recognition elements can be used as HPV/toxin sensors. A highly sensitive nanopipette probe can be precisely positioned, unlike other nanosensing technologies, at any subcellular region of a single living cell with submicron accuracy using a micromanipulator. This approach uses a movable sensor on an attached cell, in contrast to a fixed sensor detecting responses from floating cells. The functionalized nanopipette paves the way for in vivo immunoassay down to the single cell level.
Reference:
S. Umehara, M. Karhanek, R. W. Davis and N. Pourmand, PNAS, 2009, 106, 4611.

3126-Pos
The Nano-Scale Secret of Biological Secretion for Adhesion
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We discovered, by employing the atomic force microscope and transition electron microscopy, that 1) ivy and marine mussels secrete nanoparticles for surface permanent adhesion, whose force is significant larger than the force generated by gecko for reversible surface adhesion; and 2) Sundew secretes nanoparticles, which are cross-linked with muco-polysaccharide for high elastic adhesive for prey trapping. The nanoparticle-based adhesion mechanism has important implications for engineering surface adhesive materials and devices for biomedical applications.
Adhesion in nature has been the focus of intense study over the past few years. Nevertheless, research in this field has primarily concentrated on understanding the chemical aspects of adhesion. While scientists have been able to determine some of the molecular structures present in the adhesives secreted by surface affixing biological systems, such as mussels and barnacles, the fundamental adhesion mechanisms used by these systems are still unknown. This research focuses on the nano-scale morphological similarities of adhesive materials secreted from marine mussels, barnacles and ivy. We have discovered that marine mussels secrete large amounts of adhesive materials in the form of nanoparticles for surface adhesion. This is in keeping with our previous work, which indicated a similar phenomenon for ivy. Both studies concur with earlier research on marine barnacles, polychaetes and sea stars. Taken together, these results indicate that nanoparticles are used by natural, biological systems to increase surface adhesion.
We recently extended the study to Sundew and observed that nanoparticles secreted from the Sundew tentacles form scaffolds by cross-linking with munopolysaccharide. The secreted material is highly elastic and has been effectively used by the sundew for trapping prey.
The ivy nanoparticles have been isolated from the secretion using SEC-HPLC. Physical properties have been further characterized and will be discussed in details through this talk.

Biotechnology & Bioengineering II

3127-Pos
A Novel Approach for Efficient Photosynthetic Hydrogen Production
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Despite its enormous complexity, the plant PSI is arguably the most efficient nano-photochemical machine in Nature. It emerged as a homodimeric structure containing several chlorophyll molecules over 3.5 billion years ago, and has perfected its photoelectric properties ever since. Based on the structure of cyanobacterial and plant PSI together with the information gained from recently discovered PSI encoded by marine viruses we suggested a holistic solution for reasonable and efficient photosynthetic hydrogen production. Essentially what separates photosynthesis and respiration is the unique soluble cytochrome recognition by cytochrome oxidize and PSI. We think that the virus eliminated it by the introduction of PsaJ-F fusion protein. This generates a novel photorespiration that can be operated under anaerobic conditions providing there is an electron acceptor available. Thus practically we can utilize it for hydrogen production from organic material where the electron is donating by PSI > -0.6 V instead of NADH -0.34 V where PSII is inactivated and that way to separate for the first time in photosynthetic organism oxygen and hydrogen production in the light. Thus our system utilizes cyanobacteria engineered to have a novel

photorespiration where PSI substitutes cytochrome oxidase and PSII temperature sensitive mutation. We suggest that for the first time our system will be able to separate oxygen evolution and efficient hydrogen production.

3128-Pos

Characterization of New and Improved Fluorescent Proteins and their Applications

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Fluorescent proteins (FPs) have become ubiquitous tools in biological and biomedical research. Since the cloning and exogenous expression of green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*, researchers have sought new variants of this and other FPs, with properties well-suited for particular imaging applications. Of special interest are FPs with different excitation and emission wavelengths, with brighter fluorescence, monomeric, and that mature rapidly at 37°C. We have examined the properties of several novel FPs isolated from fluorescent marine organisms, which were collected from the Great Barrier Reef in Australia and Belizean Barrier Reefs. All the proteins were cloned and expressed in *E. coli* and their properties were characterized. One FP, 28bc2, showed very promising characteristics having high brightness and a weak propensity to form dimers. We have modeled the structure of 28bc2 and designed mutations at the putative dimerization interface to decrease the potential to dimerize. We have further characterized the spectroscopic properties of 28bc2 wild type (wt) and the mutants and compared their properties with those of EGFP, a widely used variant of GFP. The 28bc2 wt and mutants FP are at least 2-fold brighter than EGFP and show similar pH stability profiles to that of EGFP. The photostability of 28bc2 wt and mutants is less than that of EGFP, though for some applications this is not critical. We have shown the advantages of brightness of 28bc2 mutants in one-step detection application in Western blotting and their usefulness in *in vivo* labeling demonstrated by RNA microinjection assays in zebrafish.

3129-Pos

Optimizing Functionality of Ion Channel Biosensing using Stochastic Resonance

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Stochastic resonance refers to the increased sensitivity of a system when a finite level of noise is applied to the system. This counter-intuitive concept is evidenced by a maximum in the signal-to-noise ratio with respect to applied noise level. We have applied this technique to a system of alamethicin ion channels incorporated in a planar lipid bilayer. When used as a molecular biosensor, an enhancement of the signal-to-noise ratio of such a system improves the sensor's limit of detection. Thus, by adding noise to the biosensor, we can maximize its sensitivity. We also show how this technique can be used to design an inherently optimal molecular biosensor. By varying the *lipid membrane area*, the alamethicin concentration, and applied voltage in each system, we control the level of noise internal to the system. Then, by noting the level of *external* noise that induces stochastic resonance, we inferred the level of *internal* noise that each variable introduces to the system. In doing so, we found that microphonic noise, which is introduced by the lipid membrane, most significantly influences the signature of stochastic resonance. Thus, we have shown that by tuning the membrane area to induce an optimal level of microphonic noise, one can design a molecular biosensor that inherently induces stochastic resonance.

3130-Pos

Simultaneous Recordings of Ligand-Gated Ion Channels using a 384 Planar Patch Clamp Substrate

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We have developed an apparatus that allows the simultaneous measurement of ligand-gated ion channels (LGICs) at 384 separate recording sites in parallel prior to, during and after ligand addition. Since the development of planar patch clamp recording techniques in 2002 the number of parallel recordings that could be done on LGICs has been limited to 48. Our apparatus measures cell membrane currents using the perforated patch clamp techniques on a polyimide substrate. Currents are measured using a single hole at each recording site or an array of 64 holes at each site (Population Patch Clamp or PPC, Finkel et al. 2006). PPC averages the membrane currents in the 64 cells at each recording site by measuring the ensemble current through all 64 cells using a single pair of electrodes. PPC increases the success rates by mitigating biological variability caused largely by cells not expressing the channel of interest. We present here the consistent ability to measure cell membrane currents simultaneously from all 384 sites. Data presented include LGIC recordings of GABA chloride channels, acid sensing ion channels (ASIC), and nicotinic acetylcholine (α1 nACh) receptors. In addition to the LGIC data presented we also present recordings of voltage-gated ion channels (VGICs) including Na_V, K_V and hERG channels. Pharmacological blockade of ion channel activity is also presented to validate the use of this apparatus for screening ion channel targets in a drug discovery setting.

Ref: Finkel, A. et al. (2006). J Biomol Screen **11**(5): 488-96.

3131-Pos

Planar Patch-Clamp Electrodes for Single Cell and Neural Network Studies

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The traditional glass pipette patch-clamp technique has contributed greatly to fundamental and pharmacological ion channel studies. The success of this serial technique has driven an effort to create wafer-based patch-clamp platforms using materials with inferior dielectric properties than glass and/or using exotic processing techniques to avoid the difficulties inherent to parallel processing of glass. We have developed a material processing scheme that generates ultrasmooth, high aspect ratio pores in fused quartz wafers. These devices are demonstrated here to be superior planar patch-clamp electrodes achieving gigaohm seals in nearly 80% of trials with a mammalian cell line, with the majority of seals over 10 GΩ and as high as 80 GΩ, competing with the best pipette-based patch-clamp measurements. Our method, amenable to batch fabrication technologies, will enable the acquisition of low noise, ion channel measurements in high throughput.

We are currently merging the abovementioned devices with voltage sensitive dye (VSD) imaging and multi-electrode array (MEA) recordings in order to study multisensory integration in the medicinal leech. Initially, the planar pores will function to provide precise placement of neurons in the leech ganglion over the MEA's. The excellent spatial resolution of the VSD's combined with the temporal resolution of MEA's will provide much information of all the neurons that respond to visual stimuli in the ganglion. Further studies may employ the planar pores as intracellular electrodes, allowing voltage control and intracellular recordings of individual neurons in the ganglion.

3132-Pos

A Two-Channel Patch-Clamp System on a Chip

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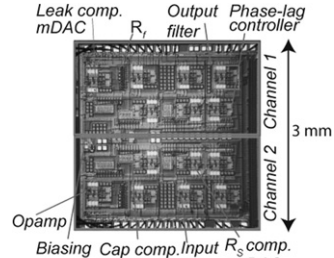
High-throughput patch-clamp systems require a large number of amplifiers in a small area. Towards a solution to this problem, we have implemented a two-channel patch-clamp system on a chip in a 3 x 3 mm area using silicon-on-sapphire (SOS) technology. The system is capable of compensating series resistances and the pipette capacitances up to 100 MΩ and 10 pF respectively. The system is able to compensate 100 % of the series resistance using phase-lag circuitry. The input-referred current noise of the system was 8 pA rms in a 10 kHz bandwidth and there was less than −40 dB of cross talk between the two channels. The power consumption of the device was 5 mW per channel. A leak compensation circuit, an input filter and an output filter were also integrated into the system. We have demonstrated the capabilities of the system by recording $Na_V1.7$ sodium currents from HEK 293 cells. This accurate, low-noise system can be used with planar electrodes to produce massively parallel high-throughput patch-clamp systems that can make recordings from 384 or more cells simultaneously.



3133-Pos

Effects of Continous Electrical Field Stimulation and Hypertrophic Stimulation on Micropatterned Cardiac Myocytes

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