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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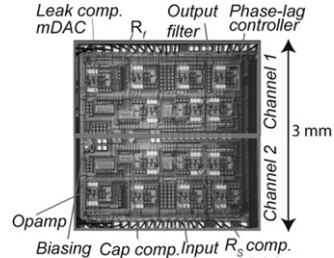
High-throughput patch-clamp systems require a large number of amplifiers in a small area. Towards a solution to this problem, we have implemented a two-channel patch-clamp system on a chip in a 3 x 3 mm area using silicon-on-sapphire (SOS) technology. The system is capable of compensating series resistances 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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