transmembrane proteins to avoid denaturation. This new capability to separate membrane-bound species near native conditions based on affinity for certain lipid phases can be used to identify intrinsic membrane raft residents and to characterize how post-translational modifications shift the affinity of analogs to a particular lipid phase.

143-Plat
Multiplexed Microfluidic Device for Bilayer Experimentation and Drug Screening Assays on Membrane Proteins
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Membrane proteins represent >50% of the targets for the development of new drugs. However, to date no appropriate platform is available for high throughput drug screening assays on these proteins. In that context, we developed a 1-plex microfluidic device for experimentation on BLMs (bilayer lipid membrane) and membrane proteins.[1] Furthermore, we have proposed a novel methodology to prepare membranes in a closed environment, the ''lipid-plug thinning'' technique, and we have validated our device for the detection of single pore-forming species.
Here, we report the multiplexing of the platform for parallel experimentation on a series of independent BLMs. The same 3-layer glass-Teflon-glass structure is employed, where the glass substrates house microchannels and the Teflon layer microfabricated apertures (15-40 μm Ø) for BLM formation. Two potential designs are investigated. In the first design, a series of independent 1-plex devices is placed in parallel. The second design resembles the structure of a fishbone, with one common channel in one glass substrate and a series of independent channels in the second glass substrate to reduce the number of reservoirs. Bilayers are formed in the multiplexed devices with a slightly modified approach where the lipid solution is introduced in one channel only, while buffer is present in the other one. Bilayer formation is successful (~75% yield) for both designs, and BLMs exhibit good properties in terms of seal resistance (>10 GΩ), and capacitance (1.35-5.01 pF for 30-μm apertures).
We are currently comparing the two designs for both automation of the membrane formation and simultaneous electrical measurements on independent BLMs. Thereafter, we will conduct experiments on pore-forming species and membrane properties using the in-house developed gramicidin-based assay.[2]
1. Stimberg et al., Proceedings MicroTAS 2010 & 2011.
2. Stimberg et al., Small, submitted.

144-Plat
Patterned Substrates for the Study of Axonal Differentiation and Neuronal Response to Smooth Microtopography
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The development of two and three-dimensional patterned substrates provides new ways to study neuronal behavior. The creation of two-dimensional (2D) patterned surfaces through the use of photolithography, microcontact printing, and self-assembled monolayer (SAM) chemistry allows for the study of axonal differentiation. Through the use of these methods, we have created starburst patterns to which E18 mouse hippocampal neurons are confined. Utilizing immunohistochemistry to specifically stain for the tau protein, predominantly localized along microtubules in the axon, we have found that neurite differentiation is not a predetermined process; rather, it is environmentally determined. The compilation of statistical data has shown that a mere 20μm difference in the distance a neurite is allowed to grow will determine polarization.
Grayscale photolithography and solvent-assisted molding (SAMo) allow us to create smooth microtopography that mimics the microtopography encountered in vivo. We have fabricated continuous three-dimensional (3D) wave patterns, varying height as well as the peak-to-peak distance, to the study of neuronal behavior in response to smooth variations in microenvironment. This will provide insights into the limitations a neuron may experience during pathfinding in vivo.

145-Plat
Red Blood Cell Sickling During Oxygen Cycles in a Microdroplet Device
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We have developed a novel microfluidic device to study repetitive sickling on individual red blood cells by replicating the physiological oxygen cycling of the vascular circulatory system (Abbyad et al., Lab Chip, 2011, 11, 813). A small number of red blood cells from sickle cell patients are encapsulated in an array of aqueous microdroplets. These microdroplets are anchored and arranged in a 2-dimensional array against the flow of the carrier oil. Precise spatial and temporal changes in oxygen concentration are obtained through gas exchange with the inert oil flowing outside the droplets. By oscillating the oxygen concentration, cycles of sickling and desickling of individual red blood cells are observed in real-time. Polarization microscopy allows for the sensitive detection of intracellular hemoglobin fibers. We observed small residual intracellular hemoglobin fibers that remain even in oxygenated conditions. Since the content of droplets in the array can be controlled, active molecules at different concentrations as well as control droplets can be measured side-by-side as they are exposed to the same environmental conditions. This was used to measure cell sickling in the presence and the absence of the anti-sickling agent glyceraldehyde. The cumulative impact of repeated sickling, such as membrane damage and cell dehydration, is believed to be central to disease pathology. We are now studying phosphatidylserine outer leaflet externalization and cell dehydration as a function of deoxygenation cycle.

146-Plat
Body Mechanics Regulate the Force Threshold for Gentle Touch Sensation in the Nematode C. Elegans
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Touch is among the least understood of our senses despite its importance in our daily lives. In the model organism *C. elegans*, gentle touch is detected by six touch receptor neurons situated in the outer shell of the animal. Force applied to the body is filtered by the outer shell (cuticle, hypodermis and body wall muscles) of the body, locally straining nearby touch receptor neuron(s) and opening mechanically-gated DEG/ENaC channels through an unknown mechanism. Previously we developed a piezoresistive cantilever force clamp system capable of applying calibrated loads to moving *C. elegans* [S.J. Park et al., Rev Sci Instr (2011), 82:043703] and showed that wild-type (N2) animals respond to forces of only 100s of nN. Further, we showed that the outer shell of the animal dominates overall body stiffness [S.J. Park et al., PNAS (2007), 104:17376]. Since the touch receptor neurons lie within the outer shell and the outer shell controls the overall mechanics of the body, we hypothesized that the force threshold for gentle touch avoidance is regulated by body stiffness. Building on our prior work showing that body stiffness can be reversibly modulated with optogenetically-induced changes in body wall muscle tone [B.C. Petzold et al., Biophys J (2011), 100:1977], we measured the force threshold for behavioral response while modulating body stiffness with Channelrhodopsin-2. In animals with hypercontracted muscles and elevated body stiffness, we found that larger forces were generally required to elicit a touch avoidance response. These findings suggest that body mechanics play an important role in filtering applied loads to the touch receptor neurons, ultimately modulating the force sensitivity of the animal, and imply that skin plays a critical role in touch sensation in both *C. elegans* and higher organisms.

Symposium: Temperature Regulation of Channels

147-Symp
Regulation of the Cold Sensor TRPM8 Channels
Tibor Rohacs.
UMDNJ - New Jersey Medical School, Newark, NJ, USA.
Transient Receptor Potential Melastatin 8 (TRPM8), channels are well established sensors of environmental cold temperatures. They can also activated by chemical agonist, such as menthol and icilin. Activation of these channels requires the presence of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]. This presentation will discuss the role this lipid plays in the regulation of TRPM8 channels upon cold and menthol activation.

148-Symp
Modular Thermal Sensors in Temperature-Gated TRP Channels
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Physiology and Biophysics, SUNY-Buffalo, Buffalo, NY, USA.
A group of ion channels in the TRP family, the so-called thermal TRP channels, exhibit unprecedentedly strong temperature dependence, some of which reach Q10 > 100 as compared to 2-3 for typical enzymatic reactions. The strong thermal sensitivity of these channels arises because their gating involves large