could be detected with co-IP experiments using the full length α-subunits. Furthermore, co-expression of Kv3.1 with the (NKv3.1)Kv6.4 chimera - in which the Kv3.1 N-terminus has been introduced in a Kv6.4 background - did also not lead to functional heterotetramers. These results together suggest that the inability of the Kv6.4 C-terminus to associate with its interaction partner inhibits the formation of functional Kv3.1/Kv6.4 heterotetramers. Indeed, FRET and co-IP experiments using N- and C-terminal fragments demonstrated that the C-terminus of Kv6.4 physically interacts with the N-terminus of Kv2.1 but not with the Kv3.1 N-terminus. This indicates that the specific Kv2.1/Kv6.4 heterotetramerization is determined by specific interactions between Kv2.1 and Kv6.4 that involve both the N- and C-termini.

**2383-Pos Board B402**

**The Intrinsically Disordered Tail of the Shaker Kv Channel is an Entropic Clock that Times its Binding to Scaffold Proteins**

Nitzan Zandany, Elhanan Magidovich, Shir Marciano, Irit Orr, Uri Abdu, **Ofer Yifrach**.
Ben Gurion University of the Negev, Beer Sheva, Israel.

The long C terminal tail of the Shaker Kv channel is responsible for scaffold protein-mediated Kv channel clustering, a process important for action potential propagation along the axon and across the synapse. Recently, we have shown that the Shaker B Kv channel tail, harboring the terminal PDZ-binding motif, is intrinsically disordered (ID) and that modification of the ID character of the tail affects PSD-95 binding, as well as channel clustering. A 'ball-and-chain'-like mechanism was suggested to describe the interaction of the channel's tail with the PDZ domains of the PSD-95 scaffold protein partner. Here, we adopt a polymer chain chemistry approach and demonstrate that systematic shortening of the intrinsically-disordered ShB-C tail results in a monotonic increase in the affinity to its PDZ partner protein. Employing surface plasmon resonance analysis, a linear correlation between the length of the Kv channel tail and the association binding energy to PSD-95 is observed. The analysis further reveals that only the association rate constant, but not the dissociation rate constant, is dependent on chain length. This assertion is further strengthened by isothermal calorimetry analysis of the interaction of PDZ domains with Kv channel tails that differ only in tail length. Thus, the ID tail of the channel controls the entropy of association whereas the PDZ binding motif at the tail's tip controls the interaction enthalpy. These results suggest that the Kv channel tail is an entropic clock that modulates the time for complex formation with the scaffold protein partner. The conceptual inter-molecular 'ball-and-chain' working model sheds new light on the role of the Shaker A alternative spliced variant that differs from the B variant only in terms of the length of the C terminal tail.

**2384-Pos Board B403**

**Hydrophobization of Glass Pipettes for use in Patch-Clamp Experiments**

**Arturo Galván**, Iván Ortega-Blake, Rosmarbel Morales-Nava.
Universidad Nacional Autónoma de México, Cuernavaca, Mexico.

Patch-clamp experiments are used to study a variety of electrophysiological responses such as single-channel recordings and channel kinetics. Although an effective and extensively used technique, there persists the argument that ions can flow not only through ion channels present in the membrane patch but also through the glass-lipid interface that the patch forms with the pipette, and even through the very thin glass tip. Another problem arises from the difficulty to obtain seals with large resistance that are stable and last for long periods of time. In this study we present an experimental protocol which has the purpose of reducing the above described problems, particularly we address the problem of the glass-lipid interface which has been described as an aqueous phase, thus allowing ion flow (1). Borosilicate glass pipettes are coated with poly(dimethylsiloxane) (PDMS) (2) through immersion and are cured with heat. PDMS is bound to the surface of the glass and, via a chemical reaction accelerated by heat, coats the surface, rendering it hydrophobic. This hydrophobic surfaces produces better seals of the membrane patch. In order to see how the hydrophobic surfaces modifies the glass-lipid interface here we compare seals made with and without the PDMS treatment and discuss their differences. We present the behavior of the seal and pipette resistances and their dependence on salt concentration in the electrolytical bath. The results indicate that the proposed treatment is very convenient for improving the patch-clamp technique, by increasing seal resistance, and therefore stability and longer lasting patches, as well as eliminating the possibility of ionic leakage.

(1) Biophysics and Structure of the Patch and the Gigaseal, Thomas M. Suchyna, Vladimir S. Markin Frederick Sachs, Biophysical Journal, Volume 97 738-747
(2) Aldrich, CAS:107517

**2385-Pos Board B404**

**Studying Voltage Dependent Proteins with Giant Unilamellar Vesicles in a "Whole Cell" Configuration**

**Matthias Garten**[1], Gilman E.S. Toombes[1,2], Sophie Aimon[1,3], Patricia Bassereau[1].
[1]Institut Curie, Paris, France, [2]National Institutes of Health, Bethesda, MD, USA, [3]Kavli Institue for Brain and Mind University of California, San Diego, CA, USA.

Biophysical characterization of voltage dependent proteins in-vivo is challenging because of the complex feedback and active regulation in living systems. One approach to overcome these difficulties is to reconstitute the proteins in bio-mimetic model systems such as Giant Unilamellar Vesicles (GUVs), solvent-free, cell-sized proteo-liposomes in which membrane composition, tension and curvature can be readily controlled. The membrane potential of a small patch of the GUV membrane can be controlled by aspirating it into a micropipette (''GUV-attached'' patch-clamp), thereby allowing measurements of protein function [1].

However, electrophysiological and fluorescence studies of the structural rearrangements mediating protein function would clearly benefit from the ability to control the membrane potential of entire, intact GUVs. For cells, electrical access to the cell interior can be obtained either by inserting a microelectrode or breaking a ''cell-attached'' membrane patch to go ''whole-cell''. Unfortunately the lack of a cytoskeleton makes GUVs far more sensitive to mechanical stresses than cells, which makes the ''whole-cell'' configuration quite challenging.

Examination of failed ''whole-cell'' GUV experiments suggested key roles for micropipette pressure and adhesion between the GUV membrane and micropipette, and by controlling these we were able to maintain the ''whole-GUV'' configuration for experimentally useful periods (tens of minutes). We will describe efforts to further optimize the ''whole-GUV'' technique, and to use it to study the effects of protein-lipid interactions and protein curvature preferences on the function of KvAP, a voltage-gated potassium channel.

[1] S. Aimon et al. Functional Reconstitution of a Voltage-Gated Potassium Channel in Giant Unilamellar Vesicles (2011), PLOSone 6:e25529

**2386-Pos Board B405**

**Structural and Functional Consequences of Replacing Glycine 77 with a L-Alanine at the KcsA Selectivity Filter**

D. Marien Cortes[1], Alain J. Labro[2], **Luis G. Cuello**[1].
[1]Texas Tech University Health Science Center, Lubbock, TX, USA, [2]University of Antwerp, Antwerp, Belgium.

In the Kv-channel superfamily, an allosteric mechanism between the activation gate (AG) and their selectivity filter (SF) triggers the collapse of the later, which has been the distinctive structural change associated to c-type inactivation (1). Previously, we have shown that the 2nd Threonine (T) within ''the signature sequence of K+ channels'' is a key component in the cross talk between AG and SF. In KcsA, the mutant T75A obliterates the S4 K+ binding site and halt the collapse of the SF associated to c-type inactivation. In the present work we aimed to determine the role of the S3 K+ binding site by making the G77A mutant. We have solved the X-ray structures of the G77A mutant in the closed (C) and open (O) states at 1.5 and 2.0 Å resolution, respectively. In the (C) state the SF remains in the conductive conformation and the backbone carbonyl group of Valine 76 is flipped away from the axis of symmetry and as a consequence there is not a K+ ion found at the S3 binding site. In the O state, the SF also remains in the conductive conformation albeit of having K+ ions only at the S1 and S4 binding sites, reminiscent to the ion configuration adopted by KcsA in the c-type inactivated state. The present structural analysis strongly suggests that a ''collapsed filter'' is not an essential requisite for a K+ channel achieves an inactive ion configuration, with two consecutive ion vacancies at the SF. A comprehensive functional analysis will be presented in addition to the structural studies.

1. Cuello, L. G. et al. (2010) Nature

Funding: NIH: 1R01GM097159-01A1 and Welch Foundation: BI-1757

**2387-Pos Board B406**

**S3-S4 Loop Modulates Voltage Sensing Domain Relaxation**

**W. Everett Fox**, Carlos A. Villalba-Galea.
Virginia Commonwealth University, Richmond, VA, USA.

Following activation, S4-based voltage sensing domains (VSD) undergo a voltage independent transition, known as relaxation, shifting their voltage dependence to more negative potentials. The molecular determinants for relaxation remain unknown. However, studies on Ci-VSP suggest that relaxation likely involves secondary and tertiary structure rearrangements within the VSD. Since