

data. Overall, our results suggest that NaChBac achieves selectivity for Na by a unique molecular strategy.

### 3069-Plat

#### Voltage Gated Sodium Channels - Nav Assay Formats Using Both Giga-Seal Single Cell and Ensemble Recording Modalities

Cristian Ionescu-Zanetti.

Fluxion Biosciences, South San Francisco, CA, USA.

Voltage gated sodium channels (Nav X.X) are important ion channel drug targets, due to their involvement in a number of channelopathies, as well their impact cardiac safety. Fast desensitization and voltage dependent pharmacology for these targets pose challenges for automated patch clamp instrumentation. In this study we present a comparison between single cell Giga-ohm seal data and ensemble recordings from sets of 20-cells. A number of different recording protocols, along with pharmacology and kinetic data serve demonstrate the capability of a next generation microfluidic patch clamp assay in addressing the important target class. Data from Nav 1.8 cells that traditionally exhibit low current amplitudes in endogenous systems is presented along with data from other Nav targets. Both traditional pharmacology measurements and use dependent block protocols using repeated voltage stimulation are compared to the literature.

## Platform: Membrane Fusion

### 3070-Plat

#### Influence of Salt Bridges in the Avian Influenza Virus HA on Acid-Induced Membrane Fusion and Pathogenicity

Caroline Mair<sup>1</sup>, P. Sivaramakrishna Rachakonda<sup>2</sup>, Thomas Korte<sup>1</sup>, Andreas Herrmann<sup>1</sup>.

<sup>1</sup>Humboldt University Berlin, Berlin, Germany, <sup>2</sup>German Cancer Research Center, Berlin, Germany.

The influenza virus hemagglutinin (HA) is known to play an essential role in virus infection. It is involved in cell receptor binding and uptake by endocytosis and, after internalization and trafficking to early and late endosomes, induces membrane fusion leading to the release of the viral genome into the cell.

This fusion process is mediated by a water-driven "spring-loaded mechanism" which is initiated by protonation of the HA1 globular heads in the acidic environment of the endosome. It has been shown for the HA of influenza virus X-31 that the pH of fusion can be shifted to higher or lower pH values by introducing or deleting salt bridges in the ectodomain of the glycoprotein. In particular, a mutation at the distal end of HA1 leading to the introduction of a salt bridge between two monomers significantly enhanced its stability thereby abolishing membrane fusion. It was suggested that this mutation is also involved in the development of new human pathogenic H5N1 lineages. We hypothesize that salt bridges in the HA1 ectodomain regulate replication efficiency and pathogenesis.

As a proof of principle recombinant virus particles containing the wild type or mutant HA of X-31 strain were produced and assayed by fluorescence dequenching. Fusion activity of mutant HA was significantly reduced with increasing pH compared to the wild type, suggesting an influence of the salt bridge on viral fusion. We will apply these results on avian HA and measure virus-endosome fusion by real time imaging inside living cells. Using human and avian cells we will be able to observe possible variations in endosomal pH and in membrane fusion activity.

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### 3071-Plat

#### Inhibition of Human Immunodeficiency Virus Endocytosis does not Allow its Fusion with the Cell Plasma Membrane

Michelle de la Vega<sup>1</sup>, Mariana Marin<sup>1</sup>, Naoyuki Kondo<sup>1</sup>, Kosuke Miyauchi<sup>2</sup>, Gregory Melikian<sup>1</sup>.

<sup>1</sup>Emory University, Atlanta, GA, USA, <sup>2</sup>RIKEN Yokohama Institute, Yokohama City, Japan.

We have recently shown that HIV enters target cells by fusing with endosomes, whereas its fusion with the plasma membrane does not proceed beyond the lipid mixing step. However, the mechanism of restriction of the HIV fusion at the cell surface and/or the factors that aid the virus entry from endosomes remain unclear. Here, we examined the HIV entry into a panel of target cells lines and into primary CD4<sup>+</sup> T cells. Kinetic measurements of fusion combined with time-resolved single virus imaging further supported the notion that HIV enters the cells *via* endocytosis and fusion with endosomes. To determine whether there is a block for HIV fusion at the cell surface, we attempted to redirect

fusion to the plasma membrane. Two experimental strategies were employed. First, fusion was synchronized by pre-incubating the viruses with cells at room temperature to allow CD4 and coreceptors engagement while preventing the virus uptake or fusion. However, subsequent shift to 37°C triggered quick virus uptake followed by endosomal fusion without allowing direct fusion at the cell surface. An alternative approach to redirect HIV-1 fusion was through blocking its endocytosis by a small-molecule dynamin inhibitor, dynasore. Treatment with dynasore resulted in transfer of viral lipids to the plasma membrane and virus inactivation without any detectable release of the viral content into the cytosol. Thus, in spite of the extended window of opportunity for entering from the cell surface, HIV-1 fusion failed to progress beyond the lipid mixing step. Our results further support the notion that HIV enters disparate cell types through endocytosis and fusion with endosomes. This work has been supported by the NIH GM054787 grant.

### 3072-Plat

#### Snare Mediated Vesicle Fusion in Supported Membranes: Comparing Synaptic Vesicles with Reconstituted Vesicles

Volker Kiessling<sup>1</sup>, Saheeb Ahmed<sup>2</sup>, Marta K. Domanska<sup>1</sup>, Matthew G. Holt<sup>2</sup>, Reinhard Jahn<sup>2</sup>, Lukas K. Tamm<sup>1</sup>.

<sup>1</sup>University of Virginia, Charlottesville, VA, USA, <sup>2</sup>Max-Planck-Institute for Biophysical Chemistry, Goettingen, Germany.

In vitro reconstitution experiments have played an essential role in a large body of research on SNARE-mediated membrane fusion. We and others have developed single vesicle assays to gain more detailed insight into the kinetics of vesicle docking and fusion. Previously, we used supported membranes in combination with total internal reflection microscopy (TIRFM) to record the docking and fusion of vesicles containing recombinant Synaptobrevin2 (Syb2) to an acceptor SNARE complex consisting of one Syntaxin 1a (Syx1a), one SNAP25 (SN25) and a short Syb2 fragment (Syb49-96) to ensure a 1:1 stoichiometry between Syx1a and SN25.

Here we report experiments in which the Syb2 containing vesicles were replaced by synaptic vesicles (SV) purified from rat brain. Docking and fusion of synaptic vesicles with supported membranes is efficient, fast (~35 ms) and SNARE dependent. The fusion kinetics obtained with different lipid environments in the supported membrane and under different salt conditions are compared to the kinetics measured with reconstituted Syb2 vesicles.

### 3073-Plat

#### How Lipid and Water Physical Chemistry May Control Fusion: Behavior of Membrane Interfaces and Fusion Peptides

Peter M. Kasson.

University of Virginia, Charlottesville, VA, USA.

Membrane fusion is an essential process in infection by enveloped viruses, synaptic exocytosis, and vesicle trafficking. As interacting membranes are brought together prior to fusion, the resulting membrane-water-membrane interface has surprising and non-bulk-like properties. Here we ask two questions regarding this membrane-membrane interface: how does the physical chemistry differ from bulk, and how do fusion peptides affect this interface. We have used atomistic molecular dynamics simulation to study these interfacial behavior at high resolution. At vesicle-vesicle interfaces prior to fusion, we observe in our simulations a distinct ordering of water between the two membranes and a concomitant slowing of dynamics. We used committor analysis to examine the role of fine water structure in fusion; holding the vesicles fixed while resampling water conformations from a solvent box substantially speeds fusion dynamics, with a small but significant effect on fusion energetics. These slowed dynamics may provide another reason for the reported propensity of fusion stalks to form around the periphery of a contact interface.

We have also examined the effect of influenza fusion peptides in bilayers and at membrane interfaces. We and others have previously hypothesized that lipid tail protrusion is a precursor to fusion stalk formation. In our simulations, influenza fusion peptides promote tail protrusion most strongly in a kinked helix conformation with the ends inserted into the hydrophobic layer. Straight helix and helical hairpin conformations occupying an interfacial position promote tail protrusion less strongly.

### 3074-Plat

#### Thermally Induced Fusion of Protein Free Lipid Vesicles

Maitane Ibarguren<sup>1</sup>, Paul H.H. Bomans<sup>2</sup>, Peter M. Frederik<sup>2</sup>, Boris Teuchov<sup>3</sup>, Alicia Alonso<sup>1</sup>, Felix M. Goni<sup>1</sup>.

<sup>1</sup>Unidad de Biofísica (CSIC-UPV/EHU), Leioa, Spain, <sup>2</sup>Laboratory for Materials and Interface Chemistry, Eindhoven, Netherlands, <sup>3</sup>Ohio State University, Columbus, OH, USA.

In an attempt to develop a minimal model system of cell membrane fusion in the absence of external catalysts we have used large unilamellar vesicles