

# Emerging Concepts in Ion Channel Biophysics

Mexico City, Mexico | October 10–13, 2017



Biophysical Journal



## **Organizing Committee**

Leon D. Islas,  
National Autonomous University of Mexico, Mexico

Froylan Gómez-Lagunas,  
National Autonomous University of Mexico, Mexico

Tamara Rosenbaum,  
National Autonomous University of Mexico, Mexico

# Thank You to Our Sponsors



October 2017

Dear Colleagues,

We would like to welcome you to the Biophysical Society Thematic Meeting, *Emerging Concepts in Ion Channel Biophysics*, co-sponsored by the Mexican government agency Consejo Nacional de Ciencia y Tecnología (CONACYT) and by the National Autonomous University of Mexico (UNAM).

The Biophysical Society thematic meetings are held in different locations around the world to provide opportunities for scientists from diverse backgrounds to get together and exchange ideas on a focused topic. Our specific meeting aims to bring together biophysicists using different approaches in the study of ion channels to help understand how these proteins are affected by their microenvironment, and to further comprehend their structure, gating properties, and function. It is our hope that you will actively participate in the discussions following each talk, the poster sessions, and the informal exchanges that will be possible during social events and free time.

This meeting also constitutes a wonderful opportunity for local scientists exploring the field of ion channel biophysics to network with renowned experts from all over the world. We are optimistic that these connections will help strengthen and grow the field in México.

The venue for *Emerging Concepts in Ion Channel Biophysics* is the Palacio de la Autonomia, UNAM, which is located in the Centro Histórico (historic center) of México City. The Center is home to an eclectic array of Mexican history and culture – from Aztec temples, ancient buildings, and museums to famous plazas, high-end restaurants, and busy shops.

Thank you for attending this meeting. We hope you enjoy all México has to offer!

The Organizing Committee

León D. Islas, National Autonomous University of Mexico, Mexico

Froylan Gómez-Lagunas, National Autonomous University of Mexico, Mexico

Tamara Rosenbaum, National Autonomous University of Mexico, Mexico

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## **GENERAL INFORMATION**

### ***Registration Hours/Information Location and Hours***

Registration will be located at the Palacio de la Autonomia, National Autonomous University of Mexico. Registration hours are as follows:

|                       |                    |
|-----------------------|--------------------|
| Tuesday, October 10   | 9:30 AM – 7:00 PM  |
| Wednesday, October 11 | 8:30 AM – 7:00 PM  |
| Thursday, October 12  | 1:00 PM – 7:00 PM  |
| Friday, October 13    | 8:30 AM – 11:30 AM |

### ***Instructions for Presentations***

#### **(1) Presentation Facilities:**

A data projector will be available in the Upper Level Auditorium. Speakers are required to bring their own laptops and adaptors. It is recommended to have a backup of the presentation on a USB drive in case of any unforeseen circumstances. Speakers are advised to preview their final presentations before the start of each session.

#### **(2) Poster Session:**

- 1) All poster sessions will be held on the lower level in the Sala de Vestigios of the Palacio de la Autonomia, National Autonomous University of Mexico.
- 2) A display board measuring 95 cm wide x 130 cm high (3.1 feet wide x 4.2 feet high) will be provided for each poster. Poster boards are numbered according to the same numbering scheme as listed in the e-book.
- 3) Posters should be set up in the morning of October 10 and removed by noon October 13. All Posters are available for viewing during all poster sessions; however, there will be formal poster presentations at the following times:

|                       |                   |                             |
|-----------------------|-------------------|-----------------------------|
| Tuesday, October 10   | 3:30 PM – 4:30 PM | Odd-numbered poster boards  |
| Tuesday, October 10   | 4:30 PM – 5:30 PM | Even-numbered poster boards |
| Wednesday, October 11 | 3:05 PM – 4:05 PM | Odd-numbered poster boards  |
| Wednesday, October 11 | 4:05 PM – 5:05 PM | Even-numbered poster boards |
| Thursday, October 12  | 3:05 PM – 4:05 PM | Odd-numbered poster boards  |
| Thursday, October 12  | 4:05 PM – 5:05 PM | Even-numbered poster boards |

- 4) During the assigned poster presentation sessions, presenters are requested to remain in front of their poster boards to meet with attendees.
- 5) All posters left uncollected at the end of the meeting will be disposed.

***Meals and Coffee Breaks***

A welcome dinner will be held in the Patio at the lower level on Tuesday, October 10 from 7:00 PM – 9:30 PM.

Coffee breaks (Wednesday and Friday) will be served in the Patio.

Snacks, beer, and wine will be served during the poster sessions at the lower level in the Sala de Vestigios.

***Smoking***

Please be advised that smoking is not permitted at the Palacio de la Autonomia, National Autonomous University of Mexico.

***Name Badges***

Name badges are required to enter all scientific sessions, poster sessions, and social functions. Please wear your badge throughout the conference.

***Contact***

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from October 10 – 13 during registration hours.

In case of emergency, you may contact the following:

Leon Islas  
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## Emerging Concepts in Ion Channel Biophysics

Mexico City, Mexico

October 10-13, 2017

### PROGRAM

#### *Tuesday, October 10, 2017*

|                     |   |                          |
|---------------------|---|--------------------------|
| 9:30 AM - 7:00 PM   | <b>Registration/Information</b>   | <b>Patio</b>             |
| 10:35 AM – 10:45 AM | Tamara Rosenbaum, National Autonomous University of Mexico<br><i>Opening Remarks</i>  |                          |
| <b>Session I</b>    | <b>Ligand-gated Channels</b><br>Eitan Reuveny, Weizmann Institute, Israel, Chair  |                          |
| 10:45 AM - 11:10 AM | Lucia Sivilotti, University College London, United Kingdom<br><i>The Activation of Glycine Channels: A Single-Channel Perspective</i>                               |                          |
| 11:10 AM - 11:35 AM | Cecilia Bouzat, Instituto de Investigaciones Bioquímicas de Bahía Blanca, Argentina<br><i><math>\alpha 7</math> Nicotinic Receptors at the Single-Channel Level</i> |                          |
| 11:35 AM - 12:00 PM | Vasanthi Jayaraman, University of Texas Medical School at Houston, USA<br><i>Glutamate Receptor Dynamics</i>  |                          |
| 12:00 PM - 1:30 PM  | <b>Lunch</b> (on own)   |                          |
| <b>Session II</b>   | <b>Ion Channels in Physiology</b><br>László Csanády, Sámuelweis University, Hungary, Chair  |                          |
| 1:30 PM - 1:55 PM   | Polina Lishko, University of California, Berkeley, USA<br><i>Ion Channels and Sperm Physiology</i>  |                          |
| 1:55 PM - 2:15 PM   | Andrea Brüggemann, Nanion Technologies GmbH, Germany *<br><i>News and Views on Cardiac Safety</i>   |                          |
| 2:15 PM - 2:40 PM   | Uhtack Oh, Seoul National University, South Korea<br><i>Physiological Implications of Anoctamin 1, a Calcium-activated Chloride Channel</i>                         |                          |
| 2:40 PM - 3:05 PM   | Andrea Meredith, University of Maryland, USA<br><i>Molecular Mechanisms of Circadian Variation in BK Channel Properties</i>   |                          |
| 3:05 PM - 3:30 PM   | Thomas Voets, University of Leuven, Belgium<br><i>TRP Channels in Noxious Heat Sensing and Pain</i>   |                          |
| 3:30 PM - 5:30 PM   | <b>Poster Session I</b>   | <b>Sala de Vestigios</b> |
| <b>Session III</b>  | <b>Keynote Talk</b><br>Leon D. Islas, National Autonomous University of Mexico, Chair   |                          |
| 5:40 PM - 6:30 PM   | Frederick Sigworth, Yale University, USA<br><i>Cryo-EM Imaging of a Voltage-gated Channel with Membrane Potential Applied</i>                                       |                          |

7:00 PM Welcome Dinner **Patio**

### ***Wednesday, October 11, 2017***

|                     |  |                          |
|---------------------|--|--------------------------|
| 8:30 AM - 7:00 PM   | <b>Registration/Information</b>  | <b>Patio</b>             |
| <b>Session IV</b>   | <b>Mechanically-activated Ion Channels</b><br>William Zagotta, University of Washington, USA, Chair  |                          |
| 9:00 AM - 9:25 AM   | Jorg Grandl, Duke University, USA<br><b><i>Magnetic Force Probing of Mechanically-activated Piezo Ion Channels</i></b>   |                          |
| 9:25 AM - 9:45 AM   | Elizabeth Heath-Heckman, University of California, Berkeley, USA*<br><b><i>Using Leeches to Discover Novel Ion Channels Involved in Mechanotransduction</i></b>  |                          |
| 9:45 AM - 10:10 AM  | Miriam Goodman, Stanford University, USA<br><b><i>Genetics and Physics of in Vivo Mechanical Activation of Ion Channels</i></b>  |                          |
| 10:10 AM - 10:30 AM | H. Peter Lu, Bowling Green University, USA*<br><b><i>Revealing NMDA Receptor Hidden Conformational Open States that Block Electric Current under Agonist Activation in Living Cells by a Novel Single-Molecule Patch-Clamp Fret Superresolution Microscopy</i></b> |                          |
| 10:30 AM - 10:50 AM | <b>Coffee Break</b>  | <b>Patio</b>             |
| <b>Session V</b>    | <b>Modulation of Ion Channels</b><br>Sharon Gordon, University of Washington, USA, Chair   |                          |
| 10:50 AM - 11:15 AM | Eitan Reuveny, Weizmann Institute, Israel<br><b><i>Regulation of Ion Channel Activity</i></b>  |                          |
| 11:15 AM - 11:40 AM | Ramón Latorre, University of Valparaiso, Chile<br><b><i>Modulation of the BK Channel by Auxiliary Subunits</i></b>   |                          |
| 11:40 AM - 01:30 PM | <b>Lunch</b> (on own)  |                          |
| <b>Session VI</b>   | <b>Methods for the Study of Ion Channels I</b><br>Andrea Meredith, University of Maryland, USA, Chair  |                          |
| 1:30 PM - 1:55 PM   | Werner Treptow, Universidade de Brasilia, Brazil<br><b><i>Binding of General Anesthetics to Ion Channels</i></b>   |                          |
| 1:55 PM - 2:20 PM   | Justin Taraska, NIH, USA<br><b><i>Imaging the Nanometer-scale Structure of the Plasma Membrane with Correlative Superresolution Light and Electron Microscopy</i></b>  |                          |
| 2:20 PM - 2:45 PM   | William Zagotta, University of Washington, USA<br><b><i>Molecular Mechanisms of Regulation of Ion Channels by Intracellular Domains</i></b>  |                          |
| 2:45 PM - 3:05 PM   | Bruce Cohen, Lawrence Berkeley National Laboratory, USA*<br><b><i>Characterization of Dynamic Kv Channel-Toxin Structures with Voltage Clamp Spectroscopy</i></b>  |                          |
| 3:05 PM - 5:05 PM   | <b>Poster Session II</b>   | <b>Sala de Vestigios</b> |

|                    |   |
|--------------------|---|
| <b>Session VII</b> | <b>Methods for the Study of Ion Channels II</b><br>Justin Taraska, NIH, USA, Chair  |
| 5:15 PM - 5:40 PM  | Richard Aldrich, University of Texas, Austin, USA<br><i>Calcium, Calmodulin, and Potassium Channels</i>   |
| 5:40 PM - 6:05 PM  | Francisco Bezanilla, University of Chicago, USA<br><i>Optical Approaches in Studies of Excitability</i>   |
| 6:05 PM - 6:30 PM  | Ivana Nikic, Werner Reichart Centre for Integrative Neuroscience, Germany<br><i>Minimal Tags for Live-Cell Protein Labelling and Superresolution Microscopy</i>                       |
| 6:30 PM - 6:50 PM  | Corianne Van den Akker, Stanford University, USA *<br><i>Combining Electrical and Optical Measurements on Voltage-gated Potassium Channels</i>  |
| 6:50 PM - 7:10 PM  | Simon Scheuring, Weill Cornell Medicine, USA *<br><i>High-speed Atomic Force Microscopy (HS-AFM): A New Tool for the Direct Study of Conformational Changes in Gated Ion Channels</i> |

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**Thursday, October 12, 2017**


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|                     |   |                          |
|---------------------|---|--------------------------|
|                     | <b>Morning Free</b>   |                          |
| 1:00 PM - 7:00 PM   | <b>Registration/Information</b>   | <b>Patio</b>             |
| <b>Session VIII</b> | <b>Mechanisms I</b><br>Miriam Goodman, Stanford University, USA, Chair  |                          |
| 1:30 PM - 1:55 PM   | Feng Qin, SUNY, USA<br><i>Temperature-dependent Gating in Ion Channels</i>  |                          |
| 1:55 PM - 2:15 PM   | Andrés Jara-Oseguera, NIH, USA *<br><i>The Role of the Selectivity Filter in Gating of the TRPV1 Channel</i>                                    |                          |
| 2:15 PM - 2:40 PM   | László Csanády, Sammelweis University, Hungary<br><i>Asymmetric Movements Reveal Distinct Roles of CFTR's Two Nucleotide Binding Sites</i>      |                          |
| 2:40 PM - 3:05 PM   | Sharona Gordon, University of Washington, USA<br><i>Dynamic Regulation of TRPV1 Ion Channels</i>  |                          |
| 3:05 PM - 5:05 PM   | <b>Poster Session III</b>   | <b>Sala de Vestigios</b> |
| <b>Session IX</b>   | <b>Structure Approaches to Membrane Proteins</b><br>Richard Aldrich, University of Texas, Austin, USA, Chair                                    |                          |
| 5:15 PM - 5:40 PM   | Montserrat Samso, Virginia Commonwealth University, USA<br><i>Ryanodine Receptors: Cross Talk between Allosteric and Ligand-binding Domains</i> |                          |
| 5:40 PM - 6:00 PM   | Taylor Hughes, Case Western Reserve University, USA *<br><i>Structural Basis of the TRPV5 Channel Modulation Revealed by Cryo-EM</i>            |                          |

|                   |   |
|-------------------|---|
| 6:00 PM - 6:25 PM | Nancy Carrasco, Yale University, USA<br><i>Structure of Membrane Transporters</i>   |
| 6:25 PM - 6:50 PM | Crina Nimigean, Cornell University, USA<br><i>Gating and Ligand Modulation in Potassium Channels</i>                                      |
| 6:50 PM - 7:10 PM | Michael Grabe, University of California, San Francisco, USA *<br><i>Atomistic Insight into Lipid Translocation by a TMEM16 Scramblase</i> |

### ***Friday, October 13, 2017***

|                     |  |              |
|---------------------|--|--------------|
| 8:30 AM - 12:00 PM  | <b>Registration/Information</b>  | <b>Patio</b> |
| <b>Session X</b>    | <b>Lipids and Membrane Proteins</b><br>Crina Nimigean, Cornell University, USA, Chair  |              |
| 9:00 AM - 9:25 AM   | Andrea Alessandrini, CNR-Institute of Nanoscience, Italy<br><i>Lipid-protein Interactions in Model Membranes</i>   |              |
| 9:25 AM - 9:50 AM   | Toshinori Hoshi, University of Pennsylvania, USA<br><i>Dietary Oils and BK Channels</i>  |              |
| 9:50 AM - 10:15 AM  | Raimund Dutzler, University of Zurich, Switzerland<br><i>Mechanistic Relationships in the TMEM16 Family of Calcium-activated Chloride Channels and Lipid Scramblases</i> |              |
| 10:15 AM - 10:35 AM | <b>Coffee Break</b>  | <b>Patio</b> |
| <b>Session XI</b>   | <b>Mechanisms II</b><br>Andrés Jara-Oseguera, NIH, USA   |              |
| 10:35 AM - 11:00 AM | Yasushi Okamura, Osaka University, Japan<br><i>Coupling Mechanisms of Voltage-sensing Phosphatase</i>  |              |
| 11:00 AM - 11:20 AM | Paul DeCaen, Northwestern University, USA *<br><i>Defining ADPKD-2 Mutation Effects on Ciliary PKD2 Ion Channels</i>   |              |
| 11:20 AM - 11:35 AM | Richard Aldrich, University of Texas, Austin, USA<br><i>Closing Remarks and BJ Poster Awards Presentation</i>  |              |

\*Contributed talks selected from among submitted abstracts

# **SPEAKER ABSTRACTS**

**The Activation of Glycine Channels: A Single-Channel Perspective****Lucia Sivilotti.**

University College London, United Kingdom.

**No Abstract** **$\alpha 7$  Nicotinic Receptors at the Single-Channel Level****Cecilia Bouzat.**

Instituto de Investigaciones Bioquímicas de Bahía Blanca, Argentina.

The  $\alpha 7$  nicotinic receptor (nAChR), which is the homomeric member of the family, is involved in neurological, psychiatric and inflammatory disorders. Enhancement of  $\alpha 7$  function by positive allosteric modulators (PAMs) is a promising therapeutic strategy to improve cognitive deficits. PAMs have been classified by their macroscopic effects as type I, which enhance agonist-induced currents, and type II, which also decrease desensitization. To decipher the molecular basis underlying the different activities, we explored their effects on single-channel currents. We found that all PAMs enhance open-channel lifetime and produce episodes of successive openings of different durations. We identified the structural determinants for the allosteric action and the temperature sensitivity of potentiation by different PAMs. In addition to the homomeric  $\alpha 7$ , emerging evidence demonstrates the expression in brain of a novel heteromeric  $\alpha 7\beta 2$  receptor whose role and functional properties remain unknown. To establish its functional stoichiometry, we used two different experimental approaches, concatemeric technology and the electrical fingerprinting strategy with an  $\alpha 7$  subunit tagged with a reporter mutation. Our results, which include the first report of single  $\alpha 7\beta 2$  channels, revealed the stoichiometry of functional heteromeric receptors, the contribution of  $\beta 2$  subunit to channel kinetics and ion permeability, and the action of  $\alpha 7$  PAMs at  $\alpha 7\beta 2$ . This information is required for differentiating homomeric from heteromeric receptors in native cells, for understanding their distinct roles, and opens doors for the development of specific ligands.

## Glutamate Receptor Dynamics

**Vasanthi Jayaraman.**

University of Texas Medical School at Houston, TX, USA.

**No Abstract**

## Ion Channels and Sperm Physiology

**Polina V. Lishko**<sup>1</sup>, Nadja Mannowetz<sup>1</sup>, Melissa R. Miller<sup>1</sup>, Sam Kenny<sup>2</sup>, Ke Xu<sup>2</sup>.

<sup>1</sup>UC Berkeley, Berkeley, CA, USA, <sup>2</sup>UC Berkeley, Berkeley, CA, USA.

Ion channels control sperm cell physiology by regulating membrane potential, intracellular levels of calcium and pH: intracellular calcium stimulates sperm hyperactivated motility, whereas intracellular protons inhibit it. Steroid hormone progesterone produced by an ovulated egg, promotes calcium influx through sperm channel CatSper- an event so central for fertilization that men lacking these channels are infertile. Human CatSper is associated with membrane non-genomic receptor- serine hydrolase ABHD2- that degrades endogenous CatSper inhibitor 2-arachidonoylglycerol upon progesterone exposure. ABHD2 is ubiquitously expressed, and the pathway discovered in spermatozoa, is likely a universal pathway that defines membrane progesterone signaling in other tissues. ABHD2 prefers progesterone over most steroids, however its steroid-specificity profile provides an unexpected insight on how female reproductive cycle can regulate sperm fertility. Activation of CatSper channel upon progesterone exposure happens in less than a second, thus allowing calcium changes to propagate rapidly to achieve a concerted movement. Such signaling event is ensured by a nanodomain organization of the sperm control units that are located in close proximity to each other. The combination of superresolution imaging method (STORM) and electrophysiology helps to reveal a detailed nanodomain organization of sperm control units, as well as to understand their fine tuning and regulation.

## News and Views on Cardiac Safety

**Andrea Brüggemann**<sup>1</sup>, Sonja Stölzle-Feix<sup>1</sup>, Claudia Haarmann<sup>1</sup>, Alison Obergrussberger<sup>1</sup>, Markus Rapedius<sup>1</sup>, Tom Götze<sup>1</sup>, Søren Friis<sup>1,2</sup>, Nina Brinkwirth<sup>1</sup>, Ilka Rinke-Weiß<sup>1</sup>, Michael George<sup>1</sup>, Tim Strassmaier<sup>3</sup>, Rodolfo Haedo<sup>3</sup>, Niels Fertig<sup>1</sup>

<sup>1</sup>Nanion Technologies GmbH, Munich, Germany, <sup>2</sup>Department of Veterinary Clinical and Animal Science, University of Copenhagen, Copenhagen, Denmark, <sup>3</sup>Nanion Technologies Inc., NJ, USA

Drug induced arrhythmia was one major causes for the removal of drugs from the market. In the beginning of 2002 Step2 of the S7B – ICH Guideline was approved. It described the Non-clinical Testing Strategy; the in vitro IKr and in vivo QT assay. Since then no drugs were removed from the market due to Torsades-de-Pointes.

Today mutations in at least 15 different genes are described to cause a LQT syndrome. Most of them are encoding ion channels or their auxiliary subunits. In addition there are also drugs on the market that are IKr inhibitors, but show a low Torsade risk due to an additional inhibition of inward currents like L-Type Calcium currents.

For this reason the FDA started to direct a new initiative: The Comprehensive in Vitro Proarrhythmia Assay (CIPA). This initiative is focused on proarrhythmia (not QT prolongation) to improve specificity compared to in vitro hERG and in vivo QT studies

Here we are describing the CIPA initiative and present some first results. Details of the experimental designs will also be discussed.

## Physiological Implications of Anoctamin 1, a Calcium-activated Chloride Channel

Uhtaek Oh<sup>1,2</sup>.

<sup>1</sup>Korea Institute of Science and Technology, Seoul, South Korea, <sup>2</sup>Seoul National University, Seoul, South Korea.

Anoctamin 1 (ANO1/TMEM16A) is activated by intracellular  $\text{Ca}^{2+}$  and voltage. ANO1 is expressed in epithelia of salivary glands, pancreas, kidney, pulmonary airways, the retina, and sensory neurons. ANO1 is highly expressed in dorsal-root ganglion (DRG) neurons, suggesting a role in nociception. ANO1 is activated by heat over 44°C. ANO1 is highly co-expressed with TRPV1, a marker for nociceptors, suggesting the involvement in nociception. Ano1-deficient mice specifically in DRG neurons were generated. Adv/Ano1<sup>fl/fl</sup> mice that have a functional ablation of Ano1 mainly in DRG neurons showed reduced responses to painful heat. Thus, ANO1 plays an important role in mediating nociception in sensory neurons. Itch is an unpleasant sensation that evokes a desire to scratch. Because of high expression in nociceptors, ANO1 may be involved in itch signals. We found that ANO1 also mediates itch. Adv/Ano1<sup>fl/fl</sup> mice showed reduced scratching behaviors in response to non-histaminergic pruritic substances, but not to histaminergic pruritogens.  $\text{Cl}^-$  secretion is important for protection of intestinal epithelia. Whether CaCC plays a role for the  $\text{Cl}^-$  secretion in GI tracts is not known. When Ano1 is abolished in small and large intestines, carbachol-induced  $\text{Cl}^-$  conductance was significantly reduced in duodenum, jejunum and proximal colon. The colon of Ano1 deficient mice was edematous. Furthermore, when colitis was induced by dextran sodium sulfate (DSS), Ano1-deficient mice developed severe colitis in colon. These results clearly suggest that ANO1 plays an active role in secreting  $\text{Cl}^-$  in intestines. In addition, ANO1 plays a critical role in testosterone-induced benign prostate hyperplasia. Testosterone upregulates Ano1 transcripts because there are few androgen-response elements in the promoter region of Ano1. In addition, inhibition of ANO1 activity or downregulation of Ano1 reduced the size of testosterone-induced prostates. Thus, it is clear that ANO1 mediates testosterone-induced prostate hyperalgesia.

**Molecular Mechanisms of Circadian Variation in BK Channel Properties****Andrea Meredith.**

University of Maryland School of Medicine, Baltimore, MD, USA.

BK  $\text{Ca}^{2+}$ - and voltage-activated  $\text{K}^+$  channels ( $\text{K}_{\text{Ca}1.1}$ ) regulate excitability in a variety of cell types, distinctively tuned by several molecular mechanisms including alternative splicing, post-translational modifications, and protein partnering (accessory subunits and  $\text{Ca}^{2+}$  channels). We investigated the coordination of these mechanisms for the circadian regulation of BK current properties in the suprachiasmatic nucleus (SCN), the brain's intrinsic 'clock.' The molecular components that translate the clock mechanism into specific firing patterns during distinct time windows are just beginning to emerge. In the SCN circuit, BK channels play a central role in the dynamic regulation of excitability that produces daily oscillations in action potential firing. Our studies probe the clock-linked regulation of BK channel activity. The day versus night differences in BK current properties are generated by daily changeovers in BK's  $\text{Ca}^{2+}$  source, alternative splicing of the alpha subunit, and beta2 subunit-mediated inactivation. These mechanisms work in concert to establish BK channel gating as a biophysical switch, toggling membranes between day and night states, to contribute to the daily variation in SCN excitability that underlies circadian rhythm.

**TRP Channels in Noxious Heat Sensing and Pain****Thomas Voets.**

KU Leuven, Leuven, Belgium.

Acute pain represents a crucial alarm signal to protect us from injury. Whereas the nociceptor neurons that convey pain signals have been well-characterized, the identity of the molecular sensors responsible for detecting noxious thermal or mechanical insults remains largely elusive. Here, evidence will be presented that acute noxious heat sensing in vitro and in vivo is mediated by a set of three functionally redundant heat-activated TRP channels. Combined elimination of all three channels eliminates heat-induced pain responses, but does not affect pain responses to cold or mechanical stimuli, or thermal preference. Pharmacological inhibition of these channels has differential effects on pathological hypersensitivity and pain.

**Cryo-EM Imaging of a Voltage-gated Channel with Membrane Potential Applied****Frederick Sigworth.**

Yale University, New Haven, CT, USA.

**No Abstract**

**Magnetic Force Probing of Mechanically-activated Piezo Ion Channels****Jorg Grandl.**

Duke University, Durham, NC, USA.

In 2010, two proteins, Piezo1 and Piezo2, were identified as the long-sought molecular carriers of an excitatory mechanically activated current found in many cells. This discovery has opened the floodgates for studying a vast number of mechanotransduction processes. Over the past years, groundbreaking research has identified Piezos as ion channels that sense light touch, proprioception, and vascular blood flow, ruled out roles for Piezos in several other mechanotransduction processes, and revealed the basic structural and functional properties of the channel. However, many aspects of Piezo function remain mysterious, including how Piezos convert a variety of mechanical stimuli into channel activation and subsequent inactivation, and what molecules and mechanisms modulate Piezo function.

We asked what specific parts (domains) of Piezo channels sense mechanical stimulation. To probe Piezos with sub-molecular resolution we developed a novel approach where we label specific domains within Piezos with magnetic nanoparticles and use an external magnetic field to generate a precise mechanical force that is highly localized within the channel protein. Simultaneously, we measure Piezo activation electrophysiologically. These experiments identified two distinct domains as being mechanically sensitive and involved in channel inactivation and activation.

## Using Leeches to Discover Novel Ion Channels Involved in Mechanotransduction

**Elizabeth Heath-Heckman**<sup>1</sup>, Maurizio Pellegrino<sup>1</sup>, Diana Bautista<sup>1</sup>, Francisco F. De-Miguel<sup>2</sup>, David Weisblat<sup>1</sup>.

<sup>1</sup>University of California - Berkeley, Berkeley, CA, USA, <sup>2</sup>Universidad Nacional Autónoma de México, Mexico City, Mexico, Mexico.

Mechanotransduction, mechanisms by which cells convert mechanical stimuli into electrical activity, is a process conserved across all domains of life. Despite its importance, mechanotransduction is not well understood at a molecular level. To better characterize the genes involved, we used leeches in the genus *Hirudo* whose ventral nerve cord ganglia contain three classes of mechanosensory neurons distinguished by their responses to light touch (T cells), pressure (P), and potentially damaging stimuli (N). To determine which genes confer these behaviors, we performed RNASeq of the above cell types and two non-mechanosensory portions of the ganglion. Some of the most highly regulated transcripts correspond to ion channels already implicated in mechanosensation, such as ASIC, Trp, and CNG. However, two hyperpolarization-activated cyclic nucleotide-gated channels (HCNs) were also upregulated in P and N cells. HCNs, while important in the etiology of chronic pain, have not yet been shown to be involved in mechanosensation. The genome of *Helobdella robusta* encodes 7 HCNs in what appears to be a lineage-specific gene amplification, suggesting that the roles of HCNs in leeches may differ from, or be more specialized than, those in other animals. In situ hybridization showed at least five of these HCNs are expressed in the nerve cord ganglia in juvenile animals, one of them identical to the enrichment found in *Hirudo*. Preliminary experiments suggest that RNAi of the upregulated HCN in *Hirudo* P cells abrogates the normal hyperpolarization-induced “sag” current, suggesting it is the primary HCN in these cells. Future experiments will include using CRISPR-Cas9 to “knock-out” HCN genes in *Helobdella* and determine the effect on mechanosensation, as well as using *Hirudo* ex vivo ganglion preparations and primary cell culture to determine how the loss of HCN activity changes their response to mechanical stimuli.

**Genetics and Physics of in vivo Mechanical Activation of Ion Channels****Miriam Goodman.**

Stanford University, Stanford, CA, USA.

Ion channels are the first responders of hearing, touch, proprioception and pain. They convert the mechanical energy of sound, touch, movement, or tissue damage into neural signals. At least three classes of proteins have been linked to the mechano-electrical transduction (MeT) channels responsible for mechanosensation in mammals and invertebrates: DEG/ENaC/ASIC sodium channels, TRP cation channels, and Piezo cation channels. We are working to determine the protein partners that form native MeT channels and the physics of force transfer in skin-sensory composite tissues. Our work focuses on DEG/ENaC/ASIC channels responsible for touch sensation in *C. elegans* nematodes, leveraging genetic dissection, gene editing, cellular neurophysiology, and tissue mechanobiology. Prior work identified two pore-forming and two auxiliary subunits required to form native MeT channels in *C. elegans* touch receptor neurons (TRNs). New results emerging from our lab and others are revising this view. We are investigating DEGT-1 as a potential pore-forming subunit of native MeT channels. Like Pacinian corpuscles and other rapidly adapting tactile sensors in vertebrates, the TRNs respond to mechanical stimulation in a frequency-dependent manner. I will discuss our recent model of frequency-dependence (Eastwood et al, PNAS, 2015), experimental tests of its predictions, and implications for the expected properties of MeT channels in their native context versus the same channels reconstituted in cells or lipid bilayers.

Acknowledgements: This work represents the current and prior effort of the presenter and a research team, including Sylvia Fechner, Samata Katta, Amy L. Eastwood, Frederic Loizeau, Sung-Jin Park, Bryan Petzold, Beth L. Pruitt, Alessandro Sanzeni, Massimo Vergassola. It is/has been funded by NIH grants (R01EB006745, R01NS047715), NIH fellowships (F32NS065718 to ALE, F31NS093825 to SK), NSF fellowship (Petzold) and fellowship funding from Swiss National Science Foundation (Loizeau), Samsung Foundation (Park), and DFG (Fechner).

## **Revealing NMDA Receptor Hidden Conformational Open States that Block Electric Current under Agonist Activation in Living Cells by a Novel Single-Molecule Patch-clamp FRET Superresolution Microscopy**

Dibyendu Sasmal, Rajeev Yadav, **H. Peter Lu**.  
Bowling Green State University, Bowling Green, OH, USA.

Conformational dynamics plays a critical role in the activation, deactivation, and open-close activities of ion channels in living cells. Such conformational dynamics is often inhomogeneous and extremely difficult to be directly characterized by ensemble-averaged spectroscopic imaging or only by single channel patch-clamp electric recording methods. We have developed a new and combined technical approach, single-molecule patch-clamp FRET microscopy, to probe ion channel conformational dynamics in living cell by simultaneous and correlated measurements of real-time single-molecule FRET spectroscopic imaging with single-channel electric current recording. Our approach is particularly capable of resolving ion channel conformational change rate process when the channel is at its electrically off states and before the ion channel is activated, the so-called “silent time” when the electric current signals are at zero or background. We have probed NMDA (N-Methyl-D-Aspartate) receptor ion channel in live HEK-293 cell, especially, the single ion channel open-close activity and its associated protein conformational changes simultaneously. Furthermore, we have revealed that the seemingly identical electrically off states are associated with multiple conformational states, including the desensitized states. Based on our experimental results, we have proposed a new multistate clamshell model to interpret the NMDA receptor open-close dynamics. Technically, our new method has a great potential to provide new structure-function analysis for understanding the function, activity and mechanism of glutamate receptor ion-channels.

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**Regulation of Ion Channel Activity**

**Eitan Reuveny.**

Weizmann Institute, Israel.

**No Abstract**

**Modulation of the BK Channel by Auxiliary Subunits**

**Ramón Latorre.**

University of Valparaiso, Chile.

**No Abstract**

## Binding of General Anesthetics to Ion Channels

**Werner Treptow.**

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How anesthetics modulate ion-channel to account for endpoints of anesthesia has been reasoned in terms of two competing hypotheses. The first view points that indirect effects resulting from anesthetic partition into the membrane impact channel energetics and conductance to induce anesthesia. Alternatively, the site-direct hypothesis states that anesthetics bind channel receptors to affect protein equilibrium and function. Here, we have explored such hypotheses to study the haloether sevoflurane and its interaction to the well-understood resting-closed (R) and activated-open (A) structures of the mammalian voltage-gated potassium channel Kv1.2. Recent studies support that sevoflurane potentiates Kv1.2 in a dose-dependent manner shifting the open probability (PO) of the channel and increasing conductance. Accordingly, we have worked specifically at the theoretical reconstruction of PO curves of Kv1.2 by embodying the (i) modulation of the channel energetics by sevoflurane-induced changes of membrane lateral pressure and (ii) ligand binding. Extensive MD-simulations of the membrane-embedded R and A structures in presence of sevoflurane show spontaneous partition of the ligand in the lipid bilayer. Despite changes of membrane order parameters and lateral pressure, partition of sevoflurane was found to moderately impact PO curves as a result of minimal molecular reshaping between Kv structures. Contrasting the membrane-mediated results, molecular binding of sevoflurane to Kv structures was found to shift the voltage-dependence of the channel in agreement to measurements. Specifically, extensive docking and free-energy calculations show that sevoflurane binds structures R and A through multiple sites. Despite a similar interaction pattern against Kv structures, site-specific binding of sevoflurane is conformation dependent accounting for considerable shifts of channel equilibrium. The result is promising as the necessary condition to look forward for mechanistic explanations of anesthetic action involving direct interactions to specific ion channels in detriment of alternative mechanisms.

## Imaging the Nanometer-scale Structure of the Plasma Membrane with Correlative Superresolution Light and Electron Microscopy

**Justin Taraska**, Kem Sochacki.  
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Clathrin mediated endocytosis (CME) is the cell's primary internalization mechanism and is central for nutrient uptake, cellular signaling and homeostasis. For an endocytic vesicle to develop, dozens of unique proteins work together to recruit cargo and stabilize clathrin as a nanoscale honeycomb lattice on the membrane. Factors that associate with the lattice must then regulate the growth and curvature of the pit and finally cut the coated-vesicle free from the surface. Due to the technical difficulty of localizing proteins at the nanoscale across large areas of the cell the spatial organization of the vast and complex endocytic protein machinery at the plasma membrane is unknown. Here, with a large-scale correlative superresolution light and electron microscopy study, we map 19 key proteins involved in endocytosis. Our data provide a comprehensive molecular architecture of endocytic structures with nano-precision across cells. We discover a distinct spatial organization within clathrin coated pits; some factors localize only to the edge (eps15, fcho2, dynamin, amphiphysin, syndapin, snx9), or center of the lattice (epsin, NECAP, CALM, hip1r, receptor cargo), but several have discrete subpopulations in both regions (AP-2, dab2, stonin2,  $\beta$ 2-arrestin, intersectin). Furthermore, the presence or concentration of many factors within these zones changes during organelle maturation. We propose that endocytosis is driven by the recruitment, re-organization, and loss of proteins within these partitioned nano-scale zones. These data provide a framework for understanding the dynamic formation and regulation of endocytosis and a way forward to study the spatial organization of the plasma membrane.

**Molecular Mechanisms of Regulation of Ion Channels by Intracellular Domains****William N. Zagotta.**

University of Washington, Seattle, WA, USA.

The family of cyclic nucleotide-binding domain (CNBD)-containing ion channels includes CNG, HCN, and KCNH channels. While these channels all contain a C-linker and CNBD in their carboxy-terminal region and are structurally very similar, they are functionally quite diverse. Their ion selectivity ranges from strongly potassium selective (KCNH) to weakly potassium selective (HCN) to cation nonselective (CNG), and their voltage-dependence ranges from depolarization activated (KCNH) to hyperpolarization activated (HCN) to voltage independent (CNG). In addition, while the CNG and HCN channels are activated by the direct binding of cyclic nucleotide, the KCNH channels do not bind and are not regulated by cyclic nucleotides. Using a combination of X-ray crystallography and electrophysiology we have shown that this lack of regulation of KCNH channels by cyclic nucleotide is because the would-be binding pocket of KCNH channels is occupied by a segment of the channel itself, we call the intrinsic ligand. Furthermore, we have shown that the cyclic nucleotide-binding homology domain (CNBHD) of KCNH channels directly interacts with the amino-terminal eag domain of these channels. Recently, using a combination of transition metal ion FRET (tmFRET), patch-clamp fluorometry (PCF), and a fluorescent noncanonical amino acid (Anap), we have shown that there is a slow rearrangement of the eag domain/CNBHD interaction associated with the voltage-dependent activation of KCNH channels. This rearrangement produces a large voltage-dependent potentiation of the channel, similar to prepulse facilitation in other channels, which is thought to regulate cardiac and neuronal excitability. We propose that instead of cyclic nucleotide-regulation, the CNBHD of KCNH channels has evolved to produce voltage-dependent potentiation.

## Characterization of Dynamic Kv Channel-toxin Structures with Voltage Clamp Spectroscopy

Sebastian Fletcher-Taylor<sup>1</sup>, Parashar Thapa<sup>2</sup>, Jon T. Sack<sup>2</sup>, **Bruce E. Cohen**<sup>1</sup>.

<sup>1</sup>Lawrence Berkeley National Laboratory, Berkeley, CA, USA, <sup>2</sup>University of California Davis, Davis, CA, USA.

Ion channels are polymorphic membrane proteins whose states and transitions have been identified by electrophysiology, and whose static structures have begun to yield to X-ray and EM techniques. These structures have offered images of individual states, giving us starting points for identifying the complex and transient structural changes that give rise to channel physiology. To understand the structural changes that underlie the gating of voltage-gated K<sup>+</sup> channels, we have synthesized fluorescent channel activity probes based on the tarantula toxin guangxitoxin-1E (GxTX), and used these to image structural changes in the GxTX-Kv complex. We have synthesized chemoselective point mutants of GxTX, an inhibitory cystine knot peptide that binds selectively to Kv2 channels, and labeled them with a novel environment-sensitive far-red fluorophore, JP, whose emission is sensitive to the polarity of its surroundings. JP-GxTX fluorescence measured in live cell membranes is dependent on the labeling site on the toxin and changes in response to Kv2 voltage activation. Using spectral images from patch clamped cells (or *Voltage Clamp Spectroscopy*, VCS) we have developed curve fitting techniques constrained by regression analysis to identify structural changes of the complex, and to reveal the probability of Kv2 ion channel activation at each voltage. Emission spectra of the JP27 GxTX mutant comprise at least 2 species whose emission peaks and amplitudes vary reversibly with membrane potential. Depolarization redshifts the emission peaks of both species, suggestive of numerous channel-toxin structures and transitions to more polar environments during activation gating. These voltage-dependent spectra offer unprecedented detail of the dynamic structural changes of a channel-toxin complex in live cells. We expect VCS to reveal ion channel activity with greater structural and temporal resolution than methods relying on integrated and broadband fluorescence signals.

## Calcium, Calmodulin, and Potassium Channels

**Richard Aldrich.**

University of Texas, Austin, USA.

**No Abstract**

## Optical Approaches in Studies of Excitability

**Francisco Bezanilla.**

University of Chicago, USA.

**No Abstract**

## Minimal Tags for Live-Cell Protein Labelling and Superresolution Microscopy

**Ivana Nikić-Spiegel.**

Werner Reichardt Centre for Integrative Neuroscience (CIN), Tuebingen, Germany.

Genetic code expansion (GCE) is emerging as an important technology for *in vitro* and *in vivo* protein manipulation and labelling. In combination with click-chemistry it allows site-specific labelling of proteins with small organic dyes. This is achieved by co-translational incorporation of unnatural amino acids (UAAs) in target proteins by using tRNA/amino-acyl tRNA synthetase pairs orthogonal to the host translational machinery. In a subsequent step, unique functional groups of UAAs are labelled with functionalized dyes in ultrafast and biocompatible click-chemistry reactions. The fact that any dye can be directly attached to the target protein in a minimally invasive way is of particular importance for single molecule science and superresolution microscopy (SRM). We previously used this technology for dual-colour live-cell labelling and SRM of distinct populations of membrane proteins in mammalian cells. In our current work we use it to visualize axonal injury in neuroinflammatory diseases, such as multiple sclerosis, at a nanoscale level.

**Combining Electrical and Optical Measurements on Voltage-gated Potassium Channels**

**Corianne Van den Akker**, Steven Boxer.  
Stanford University, Stanford, CA, USA.

The structure, precise operation mechanism and dynamics of ion channels have not yet been elucidated, because linking the structure and structure changes to function has remained challenging. We are developing new methods that allow the structure of membrane proteins to be studied in a controlled, native-like environment by simultaneously probing their structure and function. We report a second-generation membrane interferometer (Ganesan et al., Proc. Natl. Acad. Sci. 106, 2008) in which electrophysiology measurements can be performed simultaneously with high-resolution fluorescence microscopy imaging.

In the membrane interferometer, a freestanding bilayer is formed over a micropore that is positioned above a reflective mirror. The mirror allows the use of Fluorescence Interference Contrast (FLIC) and Variable Incidence Angle-FLIC (VIA-FLIC) microscopy, two surface characterization techniques that precisely locate the height of fluorescent objects relative to the silicon surface with nm resolution. Freestanding lipid bilayers are formed by rupture of lipid vesicles and are stable for over 24 hrs.

We investigate the structure-function relationship of the voltage-gated potassium channel KvAP. KvAP overexpressed in *E. Coli* is detergent-free extracted and purified using polymer nanodiscs. We show reconstitution of functional KvAP in black lipid membranes and in freestanding lipid bilayers on the membrane interferometer. KvAP channels are mutated and tagged with a fluorescent label at different positions at the S3 or S4 strand.

Progress on electrical and FLIC measurements of fluorescently labeled KvAP will be described.

**High-Speed Atomic Force Microscopy (HS-AFM): A New Tool for the Direct Study of Conformational Changes in Gated Ion Channels**

Martina Rangl<sup>1,2</sup>, Yi Ruan<sup>2</sup>, Arin Marchesi<sup>2</sup>, Pierre-Jean Corringer<sup>3</sup>, Crina Nimigean<sup>1</sup>, **Simon Scheuring**<sup>1,2</sup>.

<sup>1</sup>Weill Cornell Medicine, New York, NY, USA, <sup>2</sup>INSERM, Marseille, France, <sup>3</sup>Institut Pasteur, CNRS, Paris, France.

The advent of high-speed atomic force microscopy (HS-AFM) has opened a novel research field for the dynamic analysis of single bio-molecules: Molecular motor dynamics<sup>2,3</sup> membrane protein diffusion<sup>4</sup>, assembly<sup>5</sup> and conformational changes of transporters<sup>6</sup> could be directly visualized. Further developments for buffer exchange<sup>7</sup> and temperature control<sup>8</sup> during HS-AFM operation provide breakthroughs towards the performance of dynamic structural biochemistry using HS-AFM. Here, we show the direct visualization of conformational changes of the cyclic nucleotide gated potassium channels upon ligand binding<sup>9</sup>, and of a pentameric receptor ion channel in pH-gating<sup>10</sup>.

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## Temperature-dependent Gating in Ion Channels

**Feng Qin.**

State University of New York at Buffalo, Buffalo, NY, USA.

Mammals involve specific sensory neurons for pain and thermal sensation. Recent discoveries of transient receptor potential (TRP) channels have unraveled a group of thermal TRP channels that are responsible for transduction of physiologically relevant temperatures as well as detection of chemical cues especially those correlated to thermal perception (e.g. capsaicin, the hot ingredient of chili peppers, menthol, a cooling compound from mint, and oregano, savory and thyme, the warmth-producing spices). Thermal TRP channels are directly activated by temperature and exhibit unprecedented strong temperature dependence, some of which reach a Q10 value as large as 100, as compared to a value of 2-3 for most ion channels. The strong temperature dependence of thermal channels results from a large enthalpy change between closed and open states, about five times that of ligand- or voltage-gated channels. But how and where thermal TRP channels attain the large energetic has long been a mystery. We have investigated, by unique fast temperature jumps, the heat activation of vanilloid receptors (TRPV1-4) and have explored the biophysics and molecular basis underlying temperature sensing by the channels. This lecture will present our understanding of mechanisms of temperature-dependent gating in ion channels and will discuss critical issues and challenges facing the study of thermal channels.

## The Role of the Selectivity Filter in Gating of the TRPV1 Channel

**Andres Jara-Oseguera**, Kenton J. Swartz.  
NINDS NIH, Bethesda, MD, USA.

The TRPV1 channel is a homotetrameric non-selective cation channel that functions in nociceptors as an integrator of external noxious stimuli and endogenous pro-inflammatory signaling molecules. Although the binding sites for some TRPV1 modulators have been characterized at the functional and structural level, we still don't understand how any of these stimuli influence ion conduction in this receptor. The identification and functional characterization of the regions that function as activation gates are therefore central to understanding the mechanisms of opening and closing in the TRPV1 and other related channels. The cryo-EM structures of TRPV1 in open and closed states suggested that in addition to the intracellular gate formed by the pore-lining S6 helices, the selectivity filter could also function as an activation gate. Here we set out to determine whether the selectivity filter of TRPV1 functions as a gate, while also establishing tools to probe gating-associated conformational changes in the ion conduction pathway to investigate how this channel integrates signals from distinct modulators. We have substituted cysteines along the pore and assessed their accessibility to externally applied cadmium and silver ions in both the open and the closed states in patch clamp recordings. Our data so far suggests that the selectivity filter is not an activation gate. However, it is likely that high ion occupancy in the conduction pathway when the S6 helix gate is closed limits accessibility for external cations to cysteines located intracellularly relative to the filter. Even if the open/closed conformation of the filter does not depend on channel activation level, it is possible that the filter adopts a non-conducting conformation as observed in the structure under specific conditions yet to be identified, or that it constitutes a highly dynamic region of the receptor.

## Dynamic Regulation of TRPV1 Ion Channels

**Sharona E. Gordon**, Anastasiia Stratiievska.  
University of Washington, Seattle, WA, USA.

Increased sensitivity to noxious thermal and chemical stimuli in the setting of inflammation is mediated, at least in part, by increased plasma membrane expression of the pain-transducing ion channel TRPV1. We have studied this sensitization using the prototypical inflammatory signal nerve growth factor (NGF), which acts through the receptor tyrosine kinase receptor TrkA. Although TrkA also couples to the MAPK and PLC signaling pathways, we and others have shown that its signaling through PI3K underlies the acute phase of NGF-induced sensitization of TRPV1. Furthermore, we have shown that the ARD of TRPV1 interacts directly with PI3K, but the function of the interaction and its role in sensitization were unknown. We now demonstrate that coexpression with TRPV1 potentiates the activity of PI3K in response to NGF. A fragment corresponding to the N-terminal region of TRPV1, including the ARD, was sufficient to produce the potentiation, suggesting that potentiation may involve an increase in the catalytic activity of PI3K. We are currently examining whether potentiation of PI3K is specific to the ARD of TRPV1.

**Ryanodine Receptors: Cross Talk between Allosteric and Ligand-binding Domains****Montserrat Samso.**

Virginia Commonwealth University, Richmond, VA, USA.

The ryanodine receptor (RyR) is an intracellular  $\text{Ca}^{2+}$  release channel with major roles in skeletal/cardiac muscle contraction and brain signaling. It is the largest ion channel known, consisting of four equivalent subunits of ~5,000 residues each, an S1-S6 transmembrane domain organization, and a large and highly allosteric cytoplasmic scaffold formed by ~40 interconnected domains. With a variety of inputs being sensed simultaneously in several domains, how are these integrated to control the channel's conductance? Further modulation of the RyR-mediated  $\text{Ca}^{2+}$  release comes from its supramolecular association into lattices on the endo/sarcoplasmic reticulum, from its association with FKBP, and in skeletal muscle, from its interaction with the Cav1.1 complex. The determination of RyR's atomic structure and in multiple conformations are starting to provide more solid insight into the molecular mechanisms of RyR.

Using cryoEM we analyzed the effect that binding of the high affinity, high occupancy ligand FKBP has on the conformation of the skeletal isoform of RyR, and how this could explain the higher stability of RyR-FKBP's closed state. We also analyzed the structure of the alpha-solenoid domain, encompassing > 500 residues. This allosteric domain adopts different conformations in the cardiac and skeletal muscle isoforms despite a very similar sequence. In the skeletal isoform this domain appears to direct the assembly of RyR into lattices and participate in the relay of the signal coming from the Cav.1 complex. For the cardiac isoform we see instead an intriguing cross talk between the alpha-solenoid domain and FKBP, which appears to account more dramatically for the stabilizing effect of FKBP on RyR. In summary, cryoEM enabled to unveil new mechanisms of RyR allosterism and isoform specialization.

Supported by the American Heart Association grant 14GRNT19660003 and the Muscular Dystrophy Association grant MDA352845.

**Structural Basis of the TRPV5 Channel Modulation Revealed by Cryo-EM****Taylor Hughes**

Case Western Reserve University, Cleveland, OH, USA.

Nephrolithiasis, commonly known as kidney stones, is a common disease that affects approximately 30 million Americans annually. One of the most critical risk factors is hypercalciuria, or high levels of  $\text{Ca}^{2+}$  in urine. TRPV5 is a highly  $\text{Ca}^{2+}$  selective, kidney specific TRP channel that plays a critical role in systemic calcium homeostasis. Loss or dysfunction of TRPV5 has been shown to severely increase urine  $\text{Ca}^{2+}$  levels and the occurrence of kidney stones. To this end, we have determined TRPV5 structures in apo, econazole-inhibited and lipid-modulated states by Cryo-EM. The inhibited state TRPV5 channel structure enabled us to identify the econazole-binding site and reveal an aromatic gate that occludes the  $\text{Ca}^{2+}$  permeation through the channel. Apo- and lipid-bound TRPV5 structures uncovered the endogenous lipid modulation mechanism of this kidney specific channel and we are pursuing this project further to understand how mutations in this channel affect its gating and lead to kidney stone formation.

**Structure of Membrane Transporters****Nancy Carrasco**

Yale University, New Haven, CT, USA

**No Abstract****Gating and Ligand Modulation in Potassium Channels****Crina Nimigean**

Cornell University, Ithaca, NY, USA

**No Abstract**

**Atomistic Insight into Lipid Translocation by a TMEM16 Scramblase****Michael Grabe.**

UCSF, San Francisco, CA, USA.

The TMEM16 family of membrane proteins includes both lipid scramblases and ion channels involved in olfaction, nociception, smooth muscle contraction, and blood coagulation. The crystal structure of the fungal nhTMEM16 scramblase revealed a putative mechanism of lipid transport, whereby polar and charged lipid headgroups move through the low-dielectric environment of the membrane by traversing a hydrophilic groove on the membrane-spanning surface of the protein. Here we use computational methods to explore the membrane-protein interactions involved in lipid scrambling. Fast, continuum membrane bending calculations reveal a global pattern of charged and hydrophobic surface residues that bends the membrane in a large-amplitude sinusoidal wave resulting in bilayer thinning across the hydrophilic groove. Atomic simulations support this pattern of distortion, but also reveal two lipid headgroup interaction sites flanking the groove. The cytoplasmic site nucleates headgroup-dipole stacking interactions that form a chain of lipid molecules that penetrate into the groove. In one instance, we observe a cytoplasmic lipid interdigitate into this chain, cross the bilayer, and enter the extracellular leaflet. Additional, continuum membrane bending analysis carried out on homology models of mammalian homologues reveals that these family members also bend the membrane -- even those that lack scramblase activity. Sequence alignments suggest that the lipid interaction sites are conserved in many family members, but less so in those with reduced scrambling ability. Our analysis provides insight into how large-scale membrane bending and specific protein chemistry facilitate lipid permeation in the TMEM16 family, and we hypothesize that membrane interactions may also affect ion permeation.

## Lipid-protein Interactions in Model Membranes

**Andrea Alessandrini.**

CNR-Institute of Nanoscience, Modena, Italy.

Lipids and proteins work together to ensure that the biological membrane fulfills its functions. In this teamwork, lipids can affect the activity of protein ion channels both by the action of specific interactions and, indirectly, by their physical-chemical properties. Here we will concentrate on how the lipid bilayer assembled in different model systems could affect the action of membrane proteins by means of its peculiar thermodynamic and mechanical properties. We will show that the presence of a phase coexistence state in a lipid bilayer is able to significantly affect the conductance, the open probability and the dwell times of KcsA channels. We will use different model systems (Black Lipid Membranes, Supported Lipid Bilayers) and biophysical techniques (Differential Scanning Calorimetry, Atomic Force Microscopy) to present a consistent interpretation of the single channel data obtained by the voltage-clamp technique. In particular, we will illustrate, by Atomic Force Microscopy imaging and Spectroscopy, that KcsA channels prefer to partition into the liquid disordered phase and that this phase is characterized by a mechanical softening behavior in the transition region. We will also show other examples in which it emerges that the physical properties of lipid bilayers should be included in the analysis of the effects of exogenous molecules on ion channels. These examples include the action of neurosteroids and antimicrobial peptides on model lipid bilayers. Neurosteroids are modulators of ligand gated ion channels whose mode of action probably involves a strong interaction with the lipid bilayer, whereas antimicrobial peptides are relevant host-defense peptides strongly interacting with the membrane of bacteria and fungi. In both cases we will use different model systems such as GUVs and SLBs.

## Dietary Oils and BK Channels

### Toshinori Hoshi.

University of Pennsylvania, Philadelphia, PA, USA.

Polyunsaturated omega-3 fatty acids such as docosahexaenoic acid (DHA) are nutraceuticals enriched in oily fish and are known for their diverse health-promoting properties. We have recently demonstrated that the large-conductance  $\text{Ca}^{2+}$ - and voltage-gated Slo1 (BK) channel, important in regulation of cellular electrical excitability, is a high-affinity direct target of DHA. The channels are reversibly activated by DHA with a nM-level  $\text{EC}_{50}$ , and this stimulatory action contribute to the acute hypotensive action of DHA observed in anesthetized wild-type mice but not in Slo1 knockout mice. We have manipulated the structures of the ligand DHA and the effector Slo1 BK channel, incorporating both natural and unnatural amino acids into the channel, to elucidate the atomic properties required for an optimal DHA-channel interaction. The Z-configured double bonds near the middle of the nonpolar aliphatic tail group are a critical determinant of the affinity attribute of the DHA-channel interaction. The efficacy attribute is determined mainly by the ion-dipole interaction between the electronegative carboxylic acid head group of DHA and the para-hydroxyl group of a single tyrosine residue (Tyr 318) of the channel near the ion conduction pathway. Some derivatives of DHA were found to be effective inhibitors of the channel. All together, we have defined the atomic principles of the fatty acid action on Slo1 BK channels. Our study shows that the Slo1 BK channel, more specifically the para-hydroxyl group of Tyr 318, is a promising druggable target amenable to rational drug design based on naturally occurring compounds.

## Mechanistic Relationships in the TMEM16 Family of Calcium-activated Chloride Channels and Lipid Scramblases

**Raimund Dutzler.**

University of Zurich, Zurich, Switzerland.

The TMEM16 proteins constitute a family of membrane proteins that includes lipid scramblases and Cl<sup>-</sup> channels. Both functional branches are activated by calcium acting from the intracellular side and they share a common architecture, which was defined by the structure of the lipid scramblase nhTMEM16 (1). In this protein, each subunit of the homo-dimeric protein encompasses ten transmembrane helices and structured cytosolic domains. The structural features of subunits suggest that the dimeric protein harbors two sites of catalysis that are independent with respect to activation and lipid conduction. In scramblases, the ‘subunit cavity’, a hydrophilic membrane-traversing furrow contained within each subunit that is exposed to the lipid bilayer, provides a path for polar lipid head-groups across the membrane. It contains a conserved Ca<sup>2+</sup>-binding site located within the hydrophobic core of the membrane, which regulates activation in channels and scramblases. As shown by electrophysiology, the ion channel TMEM16A contains two ion conduction pores that are independently activated by Ca<sup>2+</sup> (2). To address the question how a channel has adapted to cope with its distinct functional properties, we have determined the structure of TMEM16A by cryo-electron microscopy at 6.6 Å (3). The protein shows a similar organization to nhTMEM16, except for changes at the ‘subunit cavity’. There, the conformation of helices is altered to form an enclosed aqueous pore that is largely shielded from the membrane.

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## Coupling Mechanisms of Voltage-sensing Phosphatase

Akira Kawanabe<sup>1</sup>, Yuka Jinno<sup>1</sup>, Souhei Sakata<sup>2</sup>, **Yasushi Okamura**<sup>1</sup>.

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Biological membrane provides environment both for electrical signal and chemical signal. Phosphoinositides (PIs) are phospholipids playing roles not only in chemical signal but also in electrical signal. PIs directly regulate many types of ion channels and transporters as known in cellular events such as modulation of membrane excitability, secretion and contraction. In turn, electrical signal influences profile of lipids. Voltage-sensing phosphatase, VSP, provides one of the molecular mechanisms underlying such electrochemical coupling. VSP contains the voltage sensor domain with similar structure to that in voltage-gated ion channels. The cytoplasmic enzyme region of VSP shows remarkable structural similarity to tumor suppressor phosphatase, PTEN. VSP exhibits depolarization-activated phosphatase activity toward three species of PIs. PTEN dephosphorylates exclusively 3-phosphate of inositol ring, whereas VSP shows broader preference: it dephosphorylates both 3-phosphate and 5-phosphate. In contrast with voltage-gated ion channels where multiple voltage sensors cooperate to regulate their central pore, VSP has single voltage sensor domain that regulates the downstream enzyme. Thus VSP raises several fundamental questions; whether the coupling mechanisms from voltage sensor is shared between voltage-gated ion channels and VSP, and whether regulatory mechanisms of enzyme are shared between PTEN and VSP. In this talk, we present recent view about mechanisms of the electrochemical coupling in VSP. By labeling single amino acid by fluorescent unnatural amino acid, Anap to Ci (*Ciona intestinalis*)-VSP heterologously expressed in *Xenopus* oocyte, we show evidence that enzyme takes multiple states probably coupled with distinct states of voltage sensor. We also show recent findings of a novel critical site for coupling between the voltage sensor domain and the phosphatase domain.

**Defining ADPKD-2 Mutation Effects on Ciliary PKD2 Ion Channels**

David E. Clapham, Xiaowen Liu, **Paul DeCaen**.  
Northwestern University, Wilmette, USA.

Autosomal Polycystic Kidney Disease type 2 (ADPKD-2) results from mutations in the *Pkd2* gene that encode putative subunits of an ion channel found in the primary cilia of epithelial cells that line the collecting ducts of the kidney (pIMCD). Primary cilia are small (5-12  $\mu\text{m}$  in length) protuberances that emanate from the apical side of polarized cells. Reports using indirect methods to characterize this ion channel are conflicting and the effects of ADPKD-mutations on ciliary PKD2 channels are unknown. Using a new conditional mouse model which harbors a fluorescent cilia reporter and either conditional knockouts for PKD1 or PKD2, we have identified PKD2 as a ciliary ion channel using direct electrophysiology measurements. In collaborative effort, we published the first structure of PKD2 using cryo-EM and identified the calcium regulatory mechanism of the PKD2-related PKD2-L1 channel. Currently, there is no drug treatment to delay ADPKD-2-related kidney cyst formation. Thus we seek a rationale for potential ADPKD treatment that depends on the type of PKD2 channel effect, by characterizing functional PKD2 channel effects of ADPKD-2 mutants and mapping these sites to the PKD2 channel structure.

# POSTER ABSTRACTS

**TUESDAY, OCTOBER 10**  
**POSTER SESSION I**  
**3:30 PM – 5:30 PM**  
**Lower Level, Sala de Vestigios**

All posters are available for viewing during all poster sessions; however, below are the formal presentations for Tuesday. Presenting authors with odd-numbered poster boards should present from 3:30 PM – 4:30 PM and those with even-numbered poster boards should present from 4:30 PM – 5:30 PM. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

|   |               |                 |
|---|---------------|-----------------|
| <b>Alva Sevilla, Ernesto</b>            | <b>1-POS</b>  | <b>Board 1</b>  |
| <b>Arcos Hernández, César</b>           | <b>4-POS</b>  | <b>Board 4</b>  |
| <b>Barabash, Miraslau</b>               | <b>7-POS</b>  | <b>Board 7</b>  |
| <b>Berglund, Nils</b>                   | <b>10-POS</b> | <b>Board 10</b> |
| <b>Brenker, Christoph</b>               | <b>13-POS</b> | <b>Board 13</b> |
| <b>Castro, Hector</b>                   | <b>16-POS</b> | <b>Board 16</b> |
| <b>Cirqueira, Leonardo</b>              | <b>19-POS</b> | <b>Board 19</b> |
| <b>De Jesús-Pérez, José</b>             | <b>22-POS</b> | <b>Board 22</b> |
| <b>Eltit, Jose</b>                      | <b>25-POS</b> | <b>Board 25</b> |
| <b>Fedorenko, Olena</b>                 | <b>28-POS</b> | <b>Board 28</b> |
| <b>Fudim, Roman</b>                     | <b>31-POS</b> | <b>Board 31</b> |
| <b>Gonzalez, Marcelo</b>                | <b>34-POS</b> | <b>Board 34</b> |
| <b>Guardiani, Carlo</b>                 | <b>37-POS</b> | <b>Board 37</b> |
| <b>Torres Morales, Erika Montserrat</b> | <b>40-POS</b> | <b>Board 40</b> |
| <b>Lewis, Amanda</b>                    | <b>43-POS</b> | <b>Board 43</b> |
| <b>Mannikko, Roope</b>                  | <b>46-POS</b> | <b>Board 46</b> |
| <b>Monsalvo-Villegas, Adrian</b>        | <b>49-POS</b> | <b>Board 49</b> |
| <b>Nematian-Ardestani, Ehsan</b>        | <b>52-POS</b> | <b>Board 52</b> |
| <b>Oliver Santiago, Enrique Ismael</b>  | <b>55-POS</b> | <b>Board 55</b> |
| <b>Paiz-Candia, Bertin</b>              | <b>58-POS</b> | <b>Board 58</b> |
| <b>Salvage, Samantha</b>                | <b>61-POS</b> | <b>Board 61</b> |
| <b>Sampedro Castañeda, Marisol</b>      | <b>64-POS</b> | <b>Board 64</b> |
| <b>Segura-Covarrubias, Guadalupe</b>    | <b>67-POS</b> | <b>Board 67</b> |
| <b>Valle Reyes, Juan Sakvador</b>       | <b>70-POS</b> | <b>Board 70</b> |
| <b>Zheng, Zeyu</b>                      | <b>73-POS</b> | <b>Board 73</b> |

Posters should be set up the morning of October 10 and removed by noon October 13.

**1-POS      Board 1****Vinculin and Its Fundamental Role in Actin Bundling Formation**

**Ernesto Alva Sevilla**<sup>2,1</sup>, Andrey Krokhotin<sup>1</sup>, Nikolay V. Dokholyan<sup>1</sup>.

<sup>2</sup>University of Texas at San Antonio, San Antonio, TX, USA. <sup>1</sup>University of North Carolina at Chapel Hill, Chapel Hill, NC, USA,

Vinculin is an abundant and essential cytoskeletal protein that localizes to focal adhesions and adherent junctions. The interaction between vinculin and actin plays a pivotal role in linking transmembrane receptors to the cytoskeleton, which in turn, is important for controlling cellular force transmission, cell morphology and motility. Vinculin binds to F-actin and undergoes a conformational change that induces formation of a cryptic dimer necessary for actin filament bundling, but nature of the dimer formed remains unknown. Here, we employ computational approaches, including discrete molecular dynamic simulations, to investigate actin-induced conformational changes in the vinculin tail (Vt) domain that facilitate dimer formation and actin bundling. We find that actin engagement with Vt alters both the N-terminal helix (H1) and C-terminus of Vt, and that conformational changes within the N- and C-terminus are necessary for the formation of stable interfaces with the actin surface. We argue that this interface is important for Vt dimerization. We show that residue deletions with the C-terminus ( $\Delta$ CT1,  $\Delta$ CT2, and  $\Delta$ CT5) affect the stability of this interface, consistent with previously published experimental findings that C-terminal deletions within Vt reduce actin bundling activity. These observations are supported by additional mutagenesis data. Intriguingly, we find that the presence of tryptophan at position 912 destabilizes H1 helix and postulate that instability introduced by W912 plays an important role allowing H1 to unfold upon actin association. Experiments are in progress to test our actin-induced vinculin dimer model and examine the role of this W912 in vinculin-mediated actin bundling.

## 4-POS Board 4

**Functional Analysis of the Voltage Sensor Domain Present in the Mammalian Sperm-Specific Na<sup>+</sup>/H<sup>+</sup> Exchanger by Patch-Clamp Fluorometry of Chimeric Fluorescent Voltage Sensor**

**César Arcos Hernández**, Takuya Nishigaki.  
Instituto de Biotecnología, Cuernavaca, Morelos, Mexico.

Sperm motility is accurately regulated by several enzymes, transporters and ion channels. Some of these proteins are expressed only in spermatozoa, but the functional relationship among them is not fully understood. Sperm-specific Na<sup>+</sup>/H<sup>+</sup> exchanger (sNHE) is an essential protein for mouse sperm motility regulation and is localized in the principal piece of mammalian spermatozoa. Differently from somatic NHEs, sNHE has two predicted regulatory domains, a voltage sensor domain (VSD) and a cyclic nucleotide binding domain (CNBD), although their functionality remains to be confirmed. In the case of the VSD, it has been proposed that hyperpolarization caused by Slo3 (a sperm specific K<sup>+</sup> channel) promotes an intracellular alkalization by the sNHE through this VSD. This signal cascade is thought to be involved in sperm hyperactivation. Interestingly, there are experiments that suggest the VSD of sNHE is functional in mice but not in humans. Correlating this, the fourth transmembrane segment of the VSD of the human sNHE, but not the mouse sNHE, lacks an arginine in a critical position, which could cause the domain not to be functional. To demonstrate functional differences in the VSD of the sNHE between human and mouse, we will produce fluorescent voltage sensors (VSFPs) using Arclight, a popular VSFP, as a template and replace its VSD by that of sNHE. We will analyze the biophysical properties of the VSFPs expressed in HEK293 cells by using patch clamp fluorometry and extrapolate the function of the VSDs of human and mouse sNHE. This project is supported by PAPIIT (DGAPA IN206116).

## 7-POS Board 7

**Statistical Kinetic Theory Insights into Selective Conduction in Biological Ion Channels**

William Gibby<sup>1</sup>, Dmitry G. Luchinsky<sup>1,2</sup>, **Miraslau L. Barabash**<sup>1</sup>, Olena A. Fedorenko<sup>3</sup>, Stephen K. Roberts<sup>3</sup>, Peter McClintock<sup>1</sup>.

<sup>1</sup>Lancaster University, Department of Physics, Lancaster, United Kingdom, <sup>2</sup>SGT Inc, Greenbelt, MD, USA, <sup>3</sup>Lancaster University, Division of Biomedical and Life Sciences, Lancaster, United Kingdom.

Biological ion channels are capable of fast conduction at near to the diffusion rate, coexisting alongside highly selective conduction. In this study we investigate selectivity between ions of the same valence, in the highly charged narrow region of the pore that forms the selectivity filter (SF). Selectivity is due to the mismatch of species excess chemical potentials, with a variety of contributions including hydration, surface tension, bonding and more [1].

The SF has a constrained geometry producing a set of quantised occupancy states due to interaction with the fixed charge associated with the amino acid residues forming the SF. We have previously demonstrated in a simple kinetic and statistical model that conduction and selectivity of channels can be described by detailed analysis of the species excess chemical potentials [2,3]. This work is extended by introducing distinguishable binding sites taking into account direct site-dependent interactions. This increases our state space and allows for the full transition pathway to be investigated, alongside the effect of SF structure mutations on the permeation process. Specifically, we have compared theoretical predictions with experimental recordings of Na<sup>+</sup> and K<sup>+</sup> conduction and selectivity in NaChBac (and its mutants), to gain physical insight into selectivity and estimate the values of the excess chemical potentials [4].

We expect the results to be applicable beyond biological systems to include artificial nanopores. The research was supported by the Engineering and Physical Sciences Research Council UK (grant No. EP/M015831/1).

[1] Roux, B., et al., *Quart. Rev. Biophys.* 37.01 (2004).

[2] Gibby, W. A. T., et al., arXiv preprint arXiv:1704.03267 (2017).

[3] Luchinsky, D. G., et al., arXiv preprint arXiv:1604.05758 (2016).

[4] Kaufman, I. Kh., et al., arXiv preprint arXiv:1612.02744 (2016).

**10-POS      Board 10****Investigating Dynamics of the NMDA Receptor Using Molecular Dynamics Simulations**

**Nils Berglund**, Birgit Schiøtt.

Aarhus University, Aarhus, Jutland, Denmark.

The NMDA receptor (NMDAR) is part of the ionotropic glutamate receptor family. Present in the majority of excitatory synapses, these membrane spanning proteins form heterotetramers and are activated through binding of both glycine and glutamate, to binding sites present in the membrane adjacent ligand-binding domain. Neurotransmitter binding leads to conformational changes that open the channel region of the protein allowing an influx of  $\text{Ca}^{2+}$  in the postsynaptic neuron, leading to depolarization and signal transduction. NMDAR is essential for brain function and brain development, crucially in the areas of learning and memory formation. The dysregulation of this receptor has been implicated in a large number of neurological conditions and it is therefore a promising target in the treatment of Alzheimer's disease, epilepsy, schizophrenia, depression as well as several other conditions.

The objective of our work was to improve the understanding of the dynamic nature of NMDAR and the effect neurotransmitter binding had on protein dynamics. Thanks to recently released, high resolution structures from X-ray crystallography and cryo-EM studies, this protein can now be studied computationally at the atomistic level. Furthermore these structures have demonstrated that NMDAR has a highly dynamic nature with a significant number of well defined and distinct conformational states. Using molecular dynamics simulations we investigated the effects of neurotransmitter binding on full heterotetrameric NMDAR structures bound to a POPC membrane. Initial results show early stages of conformational transitions as a result of ligand binding.

**13-POS      Board 13****Non-genomic Steroid Signalling in Human Sperm**

**Christoph Brenker**, Timo Strünker.  
University of Münster, Münster, Germany.

Human sperm have to fulfil a series of demanding functions during fertilization: they navigate along various chemical and physical cues to localize the oocyte, and they break through its protective vestments by hyperactivation and acrosomal exocytosis.

These processes are controlled by changes in intracellular pH ( $\text{pH}_i$ ), membrane potential ( $V_m$ ), and intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ), mediated by a subset of unique ion channels. The sperm-specific ion channels CatSper and Slo3 serve as central signalling nodes and control  $[\text{Ca}^{2+}]_i$  and  $V_m$ . Steroids released by the oocyte complex regulate the function of these ion channels and thereby sperm behaviour.

Here we show by electrophysiological recordings from human sperm how steroids differentially affect these ion channels. Using  $\text{Ca}^{2+}$  and  $V_m$  fluorimetry we studied how this modulation of ion channel function affects sperm signalling and thereby sperm function. Moreover we identified synthetic steroidal ligands that regulate ion channel function and will discuss their mechanism of action.

**16-POS Board 16****Interacting PIP<sub>2</sub> Electrostatic Charges Account for Voltage-independent Regulation of CaV2.2 Calcium Channels**

**Hector Castro**, Karina Bermeo, Isabel Arenas, David Garcia.

Universidad Nacional Autónoma de México, Mexico, Ciudad de Mexico, Mexico.

Membrane lipids are key determinants in the regulation of voltage-gated ion channels. Phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>), a membrane phospholipid, has been studied in the regulation of voltage-gated calcium channels (VGCC). However, the nature of the voltage-independent regulation of VGCC has not been fully elucidated. The aim of this work was to investigate whether the interaction of PIP<sub>2</sub> electrostatic charges are responsible for the voltage-independent regulation of Cav2.2 channels. By using biophysical and biochemical methods, charge shielding of PIP<sub>2</sub> was performed in superior cervical ganglion (SCG) neurons of the rat. Firstly, we activated Gq/11 signaling by applying 10 μM oxotremorine-M (oxo-M), a muscarinic agonist, and measured the voltage-independent regulation by using a double-pulse protocol. We characterized the voltage-independent inhibition of the calcium current through the activation by oxo-M of a PIP<sub>2</sub>-mediated signaling pathway. To determine whether phospholipase C (PLC) activation was involved in this signaling cascade, cells were treated with U-73122, a PLCβ blocker. As expected, it reduced the muscarinic calcium current inhibition. Likewise, dialysis of 100 μM diC8-PIP<sub>2</sub> attenuated the muscarinic inhibition on calcium currents. Since PIP<sub>2</sub> hydrolysis is required to calcium current inhibition by oxo-M, we tested ATP-dependency on the recovery from muscarinic inhibition. After several oxo-M applications, we observed no differences in the current recovery by decreasing ATP concentrations, indicating that PIP<sub>2</sub> resynthesis is not involved. Finally, to test whether PIP<sub>2</sub> binds directly to the calcium channel, we used neomycin. This polycation has been shown to block electrostatic interactions of PIP<sub>2</sub> with some proteins, such as PLC and ion channels. Accordingly, neomycin reduced calcium current amplitude in a voltage-independent fashion. These data support that interacting PIP<sub>2</sub> charges underlies the voltage-independent regulation of calcium channels in SCG neurons. Supported by grants, PAPIIT: IN218016, IA206317, IV100116 and CONACyT: 255635.

**19-POS      Board 19****K2P Channels Characterization and Binding to Trichloroethanol**

**Leonardo Cirqueira**, Natalia Fidelis, Werner Treptow, Alessandra Kiametis.  
Universidade de Brasília, Brasília, Distrito Federal, Brazil.

The human two-pore domain potassium channels (K2P) is a 15 member ion channel family mostly expressed in the central nervous system. They function as leak channels, contributing with inhibitory currents to hinder neuron excitability. Studies show K2Ps play a key role in developmental syndromes, anesthesia and mental disorders, turning them into promising targets to several kinds of drugs, i.e. antipsychotics, anesthetics, antidepressants and sedatives. Recently, K2Ps crystallographic structures were published, revealing two conformations differing mainly in their M4 helix position, namely up and down. Despite these advances, there's still the need to characterize their functional states and interaction to ligands.

Trichloroethanol is a sedative used widely in pediatric and veterinary procedures. Although trichloroethanol is known to activate K2Ps its molecular mechanism remains unknown.

This work aims at assessing K2Ps' structural stability, their conduction states and at studying their interaction with trichloroethanol.

Channel equilibration and structural stability, measured by the divergence to the initial crystal structure, were done with all-atom molecular dynamics (MD) simulations. To characterize if channels are conductive, ion currents were induced in simulations with electric field. The structure ensemble generated by MD was used as receptor to trichloroethanol docking searches. Equilibrium simulations indicate channels remained stable. Simulations with electric potentials are in progress. Docking calculations showed several sites in the relevant transmembrane region of the channel. Results also indicate potential conformation-dependency for ligand interaction. More accurate binding interactions will be possible with the ongoing parametrization of trichloroethanol and ensuing free energy calculations.

Obtaining the equilibrium structures and making their structural, conformational and conductive characterizations are the first steps to following studies of K2P channels, like more sophisticated free energy calculation methods, gating mechanisms and other ligands' binding sites to these K2P.

## 22-POS Board 22

**The Calcium-activated Chloride Channel TMEM16A is Regulated by Cholesterol, Phosphatidylinositol 4,5-Bisphosphate (PIP2), and Poly-Unsaturated Fatty Acids**

José De Jesús-Pérez<sup>1</sup>, Silvia Cruz-Rangel<sup>1</sup>, Edith Espino-Saldaña<sup>2</sup>, Ataúlfo Martínez-Torres<sup>2</sup>, Criss Hartzell<sup>3</sup>, Patricia Pérez-Cornejo<sup>4</sup>, Jorge Arreola<sup>1</sup>.

<sup>1</sup>Universidad Autónoma de San Luis Potosí, San Luis Potosí, San Luis Potosí, Mexico, <sup>2</sup>Instituto de Neurobiología UNAM, Querétaro, Querétaro, Mexico, <sup>3</sup>Emory University School of Medicine, Atlanta, GA, USA, <sup>4</sup>Universidad Autónoma de San Luis Potosí School of Medicine, San Luis Potosí, San Luis Potosí, Mexico.

Fluid secretion of exocrine glands, skeletal and smooth muscle contraction and regulation of gastrointestinal contraction, are some of the physiological functions that are dependent on the Cl<sup>-</sup> efflux via the homo-dimeric calcium-activated chloride channel TMEM16A. The gating of TMEM16A results from the interaction between the binding of intracellular Ca<sup>2+</sup>, membrane depolarization, extracellular proton concentration, and permeant anions. Membrane lipids regulate proteins activity such as ionic channel. In this work, we study the regulation of TMEM16A by cholesterol, phosphatidylinositol 4,5-bisphosphate (PIP2), and poly-unsaturated fatty acids using patch clamp recordings. Cholesterol depletion from the plasma membrane with methyl- $\beta$ -cyclodextrin (M- $\beta$ CD) transiently increased TMEM16A activity and dampened the run-down whilst cholesterol enrichment with M- $\beta$ CD + cholesterol enhanced the run-down. Also, TMEM16A activity decreased by PIP2 dephosphorylation by the *Danio rerio* voltage-sensitive phosphatase. Application of diC8-PIP2 in inside-out patches avoided rundown and partially restored channel activity. Changing membrane cholesterol content slowed the current decay induced by PIP2 dephosphorylation. Finally, we found that membrane enrichment with oleic, arachidonic, docosahexaenoic, or eicosapentaenoic fatty acids diminished TMEM16A activity. These results suggest a membrane-delimited protein-lipid interaction mechanism of TMEM16A regulation. We propose that the regulation of TMEM16A by lipids would influence several physiological functions critical to mammals.

## 25-POS Board 25

**Effect of Camkii-Mediated Phosphorylation in the  $\beta_{1a}$  Subunit of the Voltage-Gated  $\text{Ca}^{2+}$  Channel.**

Dora Bodnar<sup>1</sup>, Claudio F. Perez<sup>1</sup>, **Jose M. Eltit**<sup>2</sup>.

<sup>2</sup>Virginia Commonwealth University, School of Medicine, Richmond, VA, USA. <sup>1</sup>Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA,

The  $\beta_{1a}$  subunit is part of the DHPR-RyR1 complex in skeletal muscle. This macro-protein complex transduces electrical signals in the plasma membrane into cytosolic  $\text{Ca}^{2+}$  transients, triggering muscle contraction. We showed that mutations in <sup>489</sup>QVQVLTSLRRNLSFW<sup>503</sup> C-term tail domain of  $\beta_{1a}$  hinder the conformational communication between the DHPR and the RyR1. The residue Ser<sup>501</sup> is predicted to be a phosphorylation target for various protein kinases (motif NLSFW). In this study, we evaluated the specificity of protein kinases to phosphorylate this site *in vitro* and we further evaluate its effects on calcium handling in cultured myotubes. The C-term tail of  $\beta_{1a}$  subunit, encompassing residues V<sup>485</sup> through M<sup>524</sup>, was expressed as a GST-fusion protein and subjected to *in vitro* phosphorylation by PKC, MAPK and CaMKII. Western blot analysis with an anti-phospho antibody confirmed specific phosphorylation of the C-term domain by CaMKII but not PKC or MAPK. Alanine substitution of residue S<sup>501</sup> (S501A mutation) further prevented CaMKII phosphorylation confirming S<sup>501</sup> as the phosphorylation site. The effect of phosphorylation on  $\text{Ca}^{2+}$  handling was evaluated in  $\beta_1$ -null myotubes stably transfected with  $\beta_{1a}$  subunit carrying mutation S501A. Patch-clamp analysis of wt and S501A myotubes showed small to no difference in the L-type  $\text{Ca}^{2+}$  current between both genotypes. However,  $\text{Ca}^{2+}$  imaging studies revealed a significant increase in the amplitude of depolarization-induced  $\text{Ca}^{2+}$  transient ( $\text{K}^+$  stimulation) in myotubes expressing the S501A mutation. In addition, mutant cells displayed significantly higher levels of SR  $\text{Ca}^{2+}$  content and faster rate of decay of the depolarization-induced  $\text{Ca}^{2+}$  transient response. Our findings suggest that residue S<sup>501</sup> of  $\beta_{1a}$  subunit is a substrate for CaMKII phosphorylation and that its state of phosphorylation modulates  $\text{Ca}^{2+}$  release in skeletal muscle cell, presumably altering the DHPR to RyR1 signaling.

Supported by grant NIH-1R01AR06773

**28-POS      Board 28****Effects of Fixed Charge on the Selectivity of Nachbac Channels/Mutants**

**Olena Fedorenko**<sup>1</sup>, Carlo Guardiani<sup>2</sup>, Igor Kaufman<sup>1</sup>, Dmitry G. Luchinsky<sup>1,3</sup>, William A. Gibby<sup>1</sup>, Igor Khovanov<sup>2</sup>, Peter V. McClintock<sup>1</sup>, Stephen K. Roberts<sup>1</sup>.

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The present study was conducted to apply the ionic Coulomb blockade (ICB) model to bacterial Nav channels and provide new insights into the role of the fixed charge (Qf) associated with the selectivity filter (SF). Whole-cell recordings of activity for NaChBac and mutants of different Qf were analyzed for permeability and selectivity for mono- and di-valent cations. We show that 1) increasing Qf correlates with a shift in the permeation sequence for monovalent cations to larger ionic radii (K<sup>+</sup>, Rb<sup>+</sup> and Cs<sup>+</sup>) and 2) channels with  $|Qf| \geq 8$  exhibit divalent blockade and anomalous mole fraction effect in solutions containing mixtures of Na<sup>+</sup> and Ca<sup>2+</sup>; however, mutants with Qf = -8 generated with different combinations of glutamate and aspartate in the SF exhibited markedly different cation permeation profiles. These results are interpreted using the ICB model (extended to include desolvation terms) together with structural insights from molecular dynamic modelling (MD) of the SF. MD simulations provide an interpretation consistent with the ICB prediction of a stable state for the neutralized SF and for the increased Qf mutants requiring a larger number of Na<sup>+</sup> than Ca<sup>2+</sup> ions to neutralize SF. MD simulations show that a consequence of higher sodium occupancy in the pore region results in pore closure and consequently a decrease in the sodium permeation. These predictions are confirmed by experimental observations. Research is supported by EPSRC (grants No EP/M015831/1 and EP/M016889/1).

**31-POS      Board 31****Structure Guided Transformation of a Light-activated Proton Pump into a Proton Channel****Roman Fudim**<sup>1</sup>, Michael Szczepek<sup>2</sup>, Arend Vogt<sup>1</sup>, Johannes Vierrock<sup>1</sup>, Scheerer Patrick<sup>2</sup>, Peter Hegemann<sup>1</sup>.<sup>1</sup>Humboldt Universität Berlin, Berlin, Germany, <sup>2</sup>Charité – Universitätsmedizin Berlin, Berlin, Germany.

Recently, we introduced the microbial rhodopsin from *Coccomyxa subellipsoidea* (CsR) as a versatile tool to study light-driven proton pumps under electro-chemical load. Here we present the crystal structure of the wildtype rhodopsin in dark state with a resolution of 2.1 Å. The structure revealed a unique interaction between the highly conserved Arg83 and the non-conserved Tyr14. This allowed the structure guided design of a passive symmetric proton channel at physiological conditions, by replacement of Tyr14 with a negatively charged Glu, likely causing a transient salt-bridge formation between Arg83 and Glu14 within the photocycle of the rhodopsin. With further electrophysiological and spectroscopic studies we were able to link distinct current components to photocycle intermediates and could show that pump and channel currents occur sequentially in the same molecule emphasizing the role of Arg83 in maintaining unidirectionality. These findings can be valuable for a more generalized understanding of the molecular constraints distinguishing pump from channel currents.

## 34-POS Board 34

**Role of Calcium-activated Potassium Channels in L-arginine/NO Pathway Regulation by Insulin in Human Fetal Endothelium.**

Emerita Basualto<sup>1</sup>, Susana Rojas<sup>1</sup>, Marcela Cid<sup>3</sup>, **Marcelo Gonzalez**<sup>1,2</sup>.

<sup>1</sup>University of Concepcion, Concepción, Chile, <sup>2</sup>Group of Research and Innovation in Vascular Health (GRIVAS Health), Chillan, Chile, <sup>3</sup>University of Concepcion, Concepcion, Chile.

The regulation of vascular tone of placenta is a key mechanism for an adequate nutrition of the fetus and this mechanism is regulated by paracrine and endocrine signals. Between these signals, insulin is an hormone that have a important role, especially when the fetus develops his pancreas, acting directly on endothelial cells of umbilical cord and placenta. A main mechanism for regulation of vascular tone is related with the endothelial activity of calcium-activated potassium channels (KCa). In this study we want to determine if the mechanism of relaxation induced by insulin is dependent of KCa channels. Placenta and umbilical cords were obtained from normal pregnancies for placental vascular reactivity assays and isolation of human umbilical vein endothelial cells (HUVEC). Isometric tension and placental pressure were determined through wire myography and isolated cotyledon perfusion, respectively, in vessels incubated with insulin and/or tetra ethyl ammonium (TEA, K<sup>+</sup> channels inhibitor), iberiotoxin (BKCa ihibitor) and Tram-34 (SKCa inhibitor). In HUVEC, after similar treatment, the plasma membrane polarity changes (with DiBAC4(3) dye), nitric oxide synthesis (with DAF) and L-arginine transport were determined. Insulin induces relaxation in placental vein and lower perfusion pressure in placenta, both effects were blocked with KCa channels inhibitors. In HUVEC, the stimulation of insulin on NO synthesis and L-arginine transport were decreased with iberiotoxin and Tram-34. In plasma membrane polarity, the co-incubation with insulin prevent the depolarization induced by Tram-34 and iberiotoxin. The vasodilatation induced by insulin is a mechanism that depends on L-arginine transport and NO synthesis. Our results showed that this mechanism could require a previous step of plasma membrane hyperpolarization induced by activation of BKCa or SKCa in human fetal endothelium. Supported by VRID-Enlace 216.033.108-1.0 and VRID-Asociativo 213.A84.014-1.0, Universidad de Concepción, Chile.

37-POS Board 37

**Characterizing the Permeation and Na<sup>+</sup>/Ca<sup>2+</sup> Selectivity Mechanism of NaChBac Channel: a Computational Study**

**Carlo Guardiani**, Igor Khovanov.  
Warwick University, Coventry, United Kingdom.

NaChBac was the first discovered sodium voltage dependent channel, yet computational studies are still limited due to the lack of a crystal structure. In this work a pore-only construct built using the NavMs template was investigated using unbiased Molecular Dynamics and Metadynamics. When the system is simulated in NaCl the Selectivity Filter becomes stably occupied by two ions and frequent events of access of a third ion can be observed. The Potential of Mean Force (PMF) from metadynamics shows a single deep minimum due to the presence of an energy barrier that prevents permeation by a single Na<sup>+</sup> ion. This hypothesis was confirmed by a 2D-metadynamics simulation where 2 Na<sup>+</sup> ions were biased to explore the SF. The analysis of the resulting PMF revealed a knock-on permeation mechanism involving two, but possibly also three ions. Conversely, when the channel is simulated in CaCl<sub>2</sub> the SF becomes occupied by a single ion that remains blocked inside. There are two reasons for the inability of Ca<sup>2+</sup> to permeate NaChBac. First of all, the free energy of binding of Ca<sup>2+</sup> to the SF, that we computed through the free energy perturbation approach, is 3.5 times higher than that of Na<sup>+</sup>, leading to a stronger attraction to the glutamates of the EEEE-ring. Second, using metadynamics simulations with a fixed ion and a mobile ion, we showed that a Ca<sup>2+</sup>/Ca<sup>2+</sup> knock-on mechanism can not occur due to the high repulsion energy that the resident ion exerts on a second, potentially incoming ion. Our work thus, provides an alternative scenario with respect to the traditional view of the Ca-exclusion model that postulates the inability of Ca<sup>2+</sup> to bind to the Selectivity Filter of sodium channels.

## 40-POS Board 40

**Pharmacological Effects Exerted by Polypeptide Fractions Extracted from Centruroides Noxius Venom on the Oncogenic Channel Kv10.1/Eag1**

**Erika Monserrat Torres-Morales**<sup>1</sup>, Arlet Loza-Huerta<sup>1</sup>, Fredy Coronas-Valderrama<sup>2</sup>, Arturo Hernández-Cruz<sup>1</sup>, Lourival Possani<sup>2</sup>, Arturo Picones<sup>1</sup>, Enoch Luis Baltazar<sup>2</sup>, Cesar Oliver Lara Figueroa<sup>2</sup>

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The potassium channel Ether-a-go-go-1 (Eag1, KV10.1, KCNH1) is found in 70% of tumoral cells and has been linked to processes of cell proliferation, angiogenesis, migration, survival and invasion in a variety of cancers. All this has led to propose Kv10.1 as a relevant therapeutic target. Here we report the evaluation of the action of different polypeptide fractions, isolated by HPLC from the venom of the scorpion *Centruroides noxius*, on the functional activity of KV10.1 heterologously and stably expressed in HEK293 cells. By means of automated fluorescence technology, measuring Thallium ion influx through Kv10.1, 20 different polypeptide fractions were initially screened, 2 of them were identified as inhibitory hits (Tl influx reduction > 30%) and were further re-fractionated by HPLC. The pharmacological activity of these newly isolated fractions were analyzed by manual patch-clamp electrophysiology. Outward potassium currents through Kv10.1 were measured in response to 200 ms voltage steps from -100 to +50 mV in 10 mV increments applied every 5 s and from a holding voltage of -70 mV. The polypeptide fraction named CnMt9 showed a clear dose-dependent inhibitory effect, characterized by an ID50 of 14.2 ng/ml. Conductance-voltage relationship fitted with a Boltzmann equation showed that CnMt9 shifts the half-activation voltage ( $V_{1/2}$ ) from 4.7 mV to 23.8 mV and increased the slope factor from 15.5 to 33.0 mV (control versus CnMt9 at 0.2  $\mu$ g/ml) and to  $V_{1/2} > 100$  mV and slope factor to 111.7 mV, respectively, at 2  $\mu$ g/ml; n=5 to 7 cells per condition.

Supported by CONACyT (México) grants 279820 (Laboratorio Nacional de Canalopatías and CB 240305).

**43-POS      Board 43****Transduction of Repetitive Mechanical Stimuli by Piezo Ion Channels**

**Amanda Lewis**, Alisa Cui, Malcolm McDonald, Jorg Grandl.  
Duke University, Durham, NC, USA.

Many cell types experience repetitive mechanical stimuli, including vein endothelial cells during pulsating blood flow, inner ear hair cells upon sound exposure, and skin cells and their innervating DRG neurons when a finger sweeps across a textured surface or touches a vibrating object. While mechanosensitive Piezo ion channels have been clearly implicated in sensing static touch, their roles in transducing repetitive stimulations are less clear. Here, we perform electrophysiological recordings of heterologously expressed mouse Piezo1 and Piezo2 responding to repetitive mechanical stimulations. Specifically, we stimulate channels by either stretching the membrane using a high-speed pressure clamp or by directly probing the cell with a fire-polished glass pipette driven by a piezoelectric motor. We find that Piezos can act as high-pass, low-pass, or bandpass filters, depending on the stimulus waveform and duration. We then use numerical simulations and human disease-related point mutations to demonstrate that channel inactivation is the molecular mechanism underlying frequency filtering, and find evidence for two kinetically distinct inactivated states. Finally, we show that frequency filtering is conserved in rapidly-adapting mouse DRG neurons, which serve as low-pass filters when transducing repetitive mechanical stimuli. Together, our results characterize Piezos as important components in processing complex mechanical inputs, such as vibrations and blood flow, and identify inactivation as a plastic mechanism for modulating the transduction of these stimuli.

## 46-POS Board 46

**Correlation of Functional Properties of *CLCN1* Variants with the Reported Inheritance of Myotonic Symptoms**

Karen Suetterlin<sup>1</sup>, Richa Sud<sup>1</sup>, Emma Matthews<sup>1</sup>, James Burge<sup>1</sup>, Samuel McCall<sup>1</sup>, Doreen Fialho<sup>1</sup>, Mary Sweeney<sup>1</sup>, Henry Houlden<sup>1</sup>, Stephanie Schorge<sup>2,1</sup>, Michael G. Hanna<sup>1</sup>, **Roope Mannikko**<sup>1</sup>.

<sup>1</sup>UCL Institute of Neurology, London, United Kingdom, <sup>2</sup>UCL Institute of neurology, London, United Kingdom.

Myotonia Congenita (MC) is caused by mutations in the *CLCN1* gene that encodes the muscle chloride channel ClC-1. MC is caused by loss-of-function mutations that can be dominantly or recessively inherited. Accuracy of diagnosis and genetic counselling of MC for novel missense mutations can be challenging based on genetic information alone. We performed functional characterization, as a part of diagnostic set up, for 89 ClC-1 missense variants identified in 221 probands referred to our national diagnostic centre. To assess the value of functional expression we correlated the functional data with available clinical and genetic information.

Functional properties were assessed in *Xenopus laevis* oocytes using two-electrode voltage clamp. The vast majority of variants showed properties that were either wild-type-like, full loss-of-function, or had shifted voltage dependence of activation. For two variants the voltage dependence of activation could not be described with Boltzmann function and one showed dramatically increased baseline activity. In simulated heterozygous conditions the loss-of-function variants showed either wild-type-like properties or shifted voltage dependence of activation. The correlation of functional properties with inheritance pattern of clinical symptoms was excellent. For example, almost 90% of the variants with recessive functional properties were found in families categorised as recessive. We also mapped the variants onto a model of ClC-1 to identify regions associated with a functional phenotype or an inheritance pattern. We found 42% of variants in the first half of the transmembrane region were associated with dominant inheritance versus 2% outside this region. In contrast, variants in the cytoplasmic regions were significantly more likely to be categorised as uncertain pathogenicity ( $p=0.004$ ).

49-POS Board 49

**Deciphering the Functional Impact of Pirfenidone-Induced Potentiation of L-type  $\text{Ca}^{2+}$  Channels****Adrian Monsalvo-Villegas**, Guillermo Avila.

CINVESTAV, IPN, Mexico, Mexico.

**INTRODUCTION:** A synthetic compound termed pirfenidone (PFD) is well known for its ability to prevent and/or revert the excess deposition of extracellular matrix proteins (fibrosis). Thus, it is frequently used to restore alterations thought to originate from an exacerbated fibrosis in several tissues. For example, in the mouse ventricle PFD attenuates sequels of myocardial infarction. More specifically, a previous treatment with this compound decreases the area of infarct, inhibits the inducibility of tachycardia, increases electrical conduction velocity and enlarges the ejection fraction. Interestingly, our laboratory recently reported that PFD also chronically stimulates the activity of cardiac L-type  $\text{Ca}^{2+}$  channels (LTCCs; Cardiovascular Research, 2012 96:244-54).

**OBJETIVES AND METHODS:** Using primary cultures of adult rat ventricular myocytes, we have now investigated the downstream consequences of the pirfenidone-induced potentiation of LTCCs.  $\text{Ca}^{2+}$  currents ( $I_{\text{Ca}}$ ) and transients were measured simultaneously, using the whole-cell patch-clamp technique. In addition, parallel measurements of contractility and  $\text{Ca}^{2+}$  transients were performed, in intact (non-patch-clamped) myocytes.

**RESULTS:** A chronic treatment (1-2 d) with PFD led to increases in the magnitude of  $I_{\text{Ca}}$ ,  $\text{Ca}^{2+}$  transients, percentage of cell and sarcomere shortening, and velocity of contraction and relaxation. In contrast, this drug did not alter the levels of diastolic  $\text{Ca}^{2+}$ , velocity of  $\text{Ca}^{2+}$  transient decay, sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$  content nor the gain of excitation-contraction coupling (i.e. the amount of  $\text{Ca}^{2+}$  released through ryanodine receptors, divided by  $I_{\text{Ca}}$ ).

**CONCLUSIONS:** The PFD-dependent stimulation of LTCCs up-regulates  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release, which in turn accelerates contraction (positive inotropy). The stimulated relaxation is not explained by changes in the kinetics of  $\text{Ca}^{2+}$  transient decay, suggesting a positive lusitropy that likely originates from modulation of the contractile apparatus. Clearly, PFD enhances the ventricular function, not only via anti-fibrotic mechanisms, but also by promoting electrophysiological effects.

52-POS Board 52

**Voltage-dependent Gating and K<sup>+</sup> Block in Thetwik-1 K2P Channel.****Ehsan Nematian-Ardestani**<sup>1</sup>, Marcus Schewe<sup>2</sup>, Thomas Baukrowitz<sup>2</sup>, Stephen J. Tucker<sup>1</sup>.<sup>1</sup>University of Oxford, Oxford, United Kingdom, <sup>2</sup>University of Kiel, Kiel, Germany.

Recently we have shown that nearly all K2P channels can be activated by voltage (Schewe et al, 2016 Cell 164:937-49). In that study, we also demonstrated that this voltage-dependent gating is due to a 'flux-gating' mechanism located within the selectivity filter. However, the TWIK-1 channel appeared to be an exception to this general rule because it behaves primarily like a leak channel. Here we now show that TWIK-1 can also exhibit voltage-dependent activation within the physiological voltage range, but only with non-physiological permeant ions such as Rb<sup>+</sup> and NH<sub>4</sub><sup>+</sup>. Furthermore, we show that voltage-dependent activation with K<sup>+</sup> as the permeant ion can also occur, but only at voltages beyond the physiological range. The low functional activity of TWIK-1 appears to result from a combination of different possible mechanisms including post-translational modification, rapid internalization and the existence of a hydrophobic barrier deep within the inner pore. Our results now propose an additional mechanism, namely a strong interaction of K<sup>+</sup> ions with the selectivity filter, that results in a block at physiological concentrations (IC<sub>50</sub> ~ 2.8 mM). This mechanism explains the unusual voltage-dependent activation of TWIK-1 due to a strong inhibition by K<sup>+</sup> ions within the filter. These results suggest that, like other K2P channels, TWIK-1 also possesses a voltage-dependent gate within the selectivity filter; however, this mechanism appears exquisitely sensitive to the nature of the permeant ion.

55-POS Board 55

**Mibefradil and NNC55-0396 Alkalinize the Mouse Sperm Acrosome Causing  $\text{Ca}^{2+}$  Release Possibly Involving the IP3R****Enrique Ismael Oliver Santiago**<sup>2</sup>, Alberto Darszon<sup>1</sup>.<sup>1</sup>Instituto de Biotecnología/UNAM, Cuernavaca, Morelos, Mexico, <sup>2</sup>Universidad Autonoma del Estado de Morelos, Cuernavaca, Morelos, Mexico.

$\text{Ca}^{2+}$  plays a pivotal role in fertilization participating in the main functions of mammalian sperm such as maturation, motility, and acrosome reaction (AR). The AR involves the exocytosis of the acrosomal vesicle in response to different physiological and non-physiological stimuli, and is essential for fertilization. The acrosome vesicle contains hydrolytic enzymes that allow sperm to break down extracellular glycoprotein matrixes, is an intracellular  $\text{Ca}^{2+}$  store and its luminal pH (pHa) is very acidic (pH 5.3). Its similarities with endolysosomal systems has led researchers to consider the acrosome as a lysosome-related organelle.  $\text{Ca}^{2+}$  efflux and osmoregulation are key elements of the AR, however, our knowledge about the role of pHa and the molecular identity and functional role of ion channels controlling  $\text{Ca}^{2+}$  efflux from this important organelle is limited.

Previous evidence from our laboratory indicated that Mibefradil (10 M) and NNC55-0396 (10 M), both weak bases and blockers of CatSper, a sperm specific  $\text{Ca}^{2+}$  channel, elevate pHa and cause an increase in intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) in mouse and human sperm. These blockers also induce the AR. Our findings suggested that pHa alkalization regulates acrosomal  $\text{Ca}^{2+}$  transporters that release  $\text{Ca}^{2+}$  leading to  $[\text{Ca}^{2+}]_i$  increases essential for sperm AR. Here we show that Mibefradil and NNC55-0396 trigger a Xestospongin C (1 M) sensitive  $\text{Ca}^{2+}$  release from the acrosome. These findings suggest that the IP3R present in the acrosome participates in the  $\text{Ca}^{2+}$  release from this organelle induced by its alkalization triggered by weak bases such as Mibefradil and NNC55-0396.

## 58-POS Board 58

**New Molecular Determinants of the Voltage-gated Na<sup>+</sup> Channel  $\alpha$ / $\beta$ 1 Functional Interface**

**Bertin Paiz-Candia**<sup>1</sup>, Thomas Scior<sup>1</sup>, Angel Islas<sup>2,1</sup>, Alfredo Sánchez-Solano<sup>2</sup>, Claudia Mancilla-Simbro<sup>2</sup>, Lourdes Millan-PerezPeña<sup>3</sup>, Eduardo M. Salinas-Stefanon<sup>2</sup>.

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Most of the inward Na<sup>+</sup> currents responsible for the initiation of the action potential in vertebrate excitable cells are modulated by membrane immunoglobulin-like (Ig-like) proteins called  $\beta$ 1 auxiliary Na<sup>+</sup> channel subunits. By the beginning of this century, chimeric studies on the  $\beta$ 1-induced electrophysiological modulation of Na<sup>+</sup> channels had established that it is mediated by an extracellular protein-protein interaction between the pore-forming  $\alpha$  subunit and the Ig-like domain of  $\beta$ 1. Subsequently, our group has been amidst those who investigate such phenomena at an atomistic level by computational means, i.e. via standard homology molecular modelling and by a novel “analogy approach”. In the absence of the crystal structure of the  $\beta$ 1 subunit, we predicted two intramolecular bonds involved in the modulation of the rNav1.4 channel, a disulphide and salt bridge, that were soon later observed in the crystal structure of  $\beta$ 3 (a homologue of  $\beta$ 1, with a 44% sequence identity). We have also demonstrated that  $\beta$ 1 significantly accelerates the inactivation of rNav1.4 channels, in which the intracellular inactivation particle is absent, albeit within seconds. Exhaustive, unbiased sampling and extremely low homology, “analogy modelling”, allowed the identification of two consecutive, key residues on  $\beta$ 1, whereby the double mutant: T109A N110A, we called TANA, caused a pervasive electrophysiological loss-of-function. Mutant  $\beta$ 1-TANA co-expression with rNav1.4: i) abolished the  $\beta$ 1-induced left-hand shift of the steady-state inactivation curve, ii) significantly reduced the  $\beta$ 1-induced acceleration of the inactivation and recovery from inactivation, and iii) induced a current rundown upon repetitive depolarizations at 1, 2 and 5 Hz. These results may delineate the functional mammalian Na<sup>+</sup> channel  $\alpha$ / $\beta$ 1 interface and may pave the way for structure-based drug design targeting the  $\beta$ 1 subunit, which incidentally also modulates the response to some Na<sup>+</sup> channel blockers.

**61-POS Board 61****A Role for the Sodium Channel B3 Subunit in Promoting Nav1.5 Oligomerisation.**

**Samantha C. Salvage**<sup>1</sup>, Mekdes H. Mariam Debelo<sup>1</sup>, Jennifer R. Irons<sup>1</sup>, Richard Butler<sup>4</sup>, Andrew J. Thompson<sup>3</sup>, Simone Weyand<sup>1</sup>, Christopher L. H Huang<sup>2,1</sup>, Antony P. Jackson<sup>1</sup>.  
<sup>1</sup>Biochemistry, University of Cambridge, Cambridge, United Kingdom, <sup>2</sup>Physiological laboratory, University of Cambridge, Cambridge, United Kingdom, <sup>3</sup>Pharmacology, University of Cambridge, Cambridge, United Kingdom, <sup>4</sup>Gurdon Institute, University of Cambridge, Cambridge, United Kingdom.

The cardiac voltage gated sodium channel (Nav1.5) is responsible for the rapid depolarising phase of the cardiac action potential. Nav1.5 is comprised of a pore-forming sodium ion-selective  $\alpha$  subunit together with  $\beta$  subunits. The  $\beta 3$  subunit regulates multiple aspects of Nav1.5 channel gating and trafficking behaviour, and mutations in the  $\beta 3$  subunit are implicated in cardiac pathologies such as Brugada syndrome. We have determined the atomic-resolution structure of the extracellular  $\beta 3$  immunoglobulin domain and shown that it forms trimers. Here we extend this finding to study its interaction with the Nav1.5 alpha subunit.

We transiently transfected HEK293 cells with  $\beta 3$  constructs tagged with either GFP or myc and performed chemical cross-linking, gel-filtration and proximity ligation assays (PLA). This indicated the presence of  $\beta 3$  oligomers – in particular, dimers and trimers in vivo. Furthermore, using PLA and affinity-chromatography, we show that  $\beta 3$  also enhances the cross-linking of Nav1.5 alpha subunits. Interestingly, the PLA data shows that the  $\beta 3$ -induced clustering of Nav1.5 alpha subunits occurs particularly at the plasma membrane. Using whole-cell patch-clamp electrophysiology, we assess the functional implications of these multi-subunit Nav1.5 oligomers.

## 64-POS Board 64

**Gating Modifier Toxins as Hits for Developing Blockers of Nav1.4 Sodium Channel Omega Currents: Domain I-Specific Effect of Spider Toxin Hm-3**

Roope Männikkö<sup>1</sup>, Zakhar O. Shenkarev<sup>2,3</sup>, Michael G. Thor<sup>1</sup>, Antonina A. Berkut<sup>2,3</sup>, Mikhail Yu. Myshkin<sup>2,3</sup>, Alexander S. Paramonov<sup>2</sup>, Dmitry S. Kulbatski<sup>2,4</sup>, Kuzmin Dmitry<sup>5</sup>, **Marisol Sampedro Castañeda**<sup>1</sup>, Louise King<sup>1</sup>, Emma R. Wilson<sup>1</sup>, Ekaterina N. Lyukmanova<sup>2,4</sup>, Mikhail P. Kirpichnikov<sup>2,4</sup>, Stephanie Schorge<sup>1,5</sup>, Frank Bosmans<sup>6</sup>, Michael G. Hanna<sup>1</sup>, Dimitri Kullmann<sup>1,5</sup>, Alexander A. Vassilevski<sup>2</sup>.

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Hypokalaemic periodic paralyses (HypoPP) are rare channelopathies characterized by episodes of severe muscle weakness associated with low serum potassium levels, often progressing to permanent muscle weakness with age. Depolarizing gating pore currents caused by mutations in arginine residues in the voltage-sensing domains (VSDs) of skeletal muscle voltage-gated sodium and calcium channels are the underlying molecular pathomechanism. There is currently no effective, mechanistically-based treatment for HypoPP. We present the functional characterization of an Nav1.4 channel mutant previously identified in a HypoPP patient, p.R222W. The mutation neutralizes the second S4 arginine in domain I of the channel causing gating pore currents. We tested if these currents can be inhibited by gating modifier toxin Hm-3, derived from the venom of the crab spider *Heriades melloteei*. The toxin inhibits gating pore currents (IC<sub>50</sub>=5.4µM) from channels with mutations in the second arginine in VSD-I (p.R222W and p.R222G), but not from channels with analogous mutations in VSD-II or -III. Mutations in VSD-I reduced Hm-3 gating modifier effect, confirming the domain specificity of Hm-3. NMR studies of the VSD-I of Nav1.4 with Hm-3 demonstrate electrostatic and hydrophobic interactions of the toxin with the S3b helix and S3-S4 extracellular loop. Implanting the S3-S4 helix-loop-helix of VSD-I, but not VSD-II, -III or -IV, to the corresponding location of Kv2.1 conveyed Hm-3 sensitivity to the host channel. Our data identify a novel and specific binding site for neurotoxins on the S3-S4 linker region of the VSD-I of Nav1.4 and highlight gating modifier toxins as useful hits in the development of omega current blockers for HypoPP therapy.

This work was partly funded by the Molecular and Cell Biology Program of the Russian Academy of Sciences and the UK Medical Research Council.

67-POS Board 67

**Voltage-dependent Activation of TMEM16A, a Calcium-Activated Chloride Channel****Guadalupe Segura-Covarrubias**<sup>1</sup>, Silvia Cruz-Rangel<sup>2</sup>, José J. De Jesús-Pérez<sup>2</sup>, Atáulfo Matinez Torres<sup>3</sup>, Patricia Pérez-Cornejo<sup>2</sup>, Jorge Arreola<sup>2</sup>.<sup>1</sup>Instituto Potosino de Investigación Científica y Tecnológica, San Luis Potosí, San Luis Potosí, Mexico, <sup>2</sup>Universidad Autónoma de San Luis Potosí, San Luis Potosí, San Luis Potosí, Mexico,<sup>3</sup>Instituto de Neurobiología UNAM, Querétaro, Querétaro, Mexico.

The Ca<sup>2+</sup>-activated Cl<sup>-</sup> channel (CaCC) TMEM16A is widely expressed and play relevant role in critical physiological processes. The mechanism of activation of TMEM16A is complex. It involves an increase in intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) along with membrane depolarization. In addition, highly permeant anions and protons facilitate channel gating. It has been proposed that the voltage dependence of TMEM16A result from the voltage-dependent binding of Ca<sup>2+</sup>. In this work, we show that WT TMEM16A and TMEM16A lacking the high affinity Ca<sup>2+</sup> binding site are activated by voltage in the absence of intracellular Ca<sup>2+</sup> albeit to a lower extent than Ca<sup>2+</sup>. Even so, tannic acid, specific blocker TMEM16A, inhibited the currents recorded from cells dialyzed with zero and with 0.2 μM Ca<sup>2+</sup> with the same apparent affinity indicating that tinny currents are flowing through TMEM16A. The current magnitude increased after eliminating <sup>448</sup>EAVK<sub>451</sub> located in the first intracellular loop of TMEM16A with high affinity Ca<sup>2+</sup> binding site (E702Q/E705Q) mutated. The magnitude of the currents through this channel depended on the [Cl<sup>-</sup>]<sub>e</sub> and has an anion selectivity similar to that of the wild channel. Deleting 5 glutamic acids located before <sup>448</sup>EAVK<sub>451</sub> did not significant change the voltage dependence. Based on these results we propose that TMEM16A has an intrinsic voltage dependence that is negatively regulated by the first intracellular loop. Thus, the voltage dependence TMEM16A comes from the intrinsic voltage dependence here described and from the voltage dependence of the Ca<sup>2+</sup> binding.

**70-POS      Board 70****Voltage- and Ca<sup>2+</sup>- Activated Potassium Channels as a Part of Ca<sup>2+</sup> Toolkit in Healthy T Cells and Leukemic Cell Lines.****Juan Salvador Valle Reyes**, Igor Pottosin, Georgina Valencia Cruz, Liliana Liñan Rico, Oxana Dobrovinskaya.

University of Colima, University Center for Biomedical Research, Colima, México n/a, Coyoacan, Mexico.

Potassium channels play key roles in shaping electrical signals in both excitable and non-excitable cells. Yet their impact on Ca<sup>2+</sup> entry in excitable and non-excitable cells is opposite: cessation or maintenance of Ca<sup>2+</sup> entry, respectively. In lymphocytes Ca<sup>2+</sup> entry is dominated by inward-rectifying CRAC current, which for its sustained activity requires membrane repolarization, mediated primarily by Kv and KCa channels. This Ca<sup>2+</sup> entry controls maturation, activation, differentiation and apoptosis. In the present work we analysed Kv and KCa currents in acute T leukemic (Jurkat, CEM and, MOLT) and healthy T cells. Our analysis shows moderate but significant differences in Kv (Kv1.3 in all cases) channels' activation and inactivation parameters. KCa currents were represented by KCa3.1 channels in all cases except Jurkat cells, where they were mediated by KCa2.2 channels. Strikingly, Jurkat cells, which over 50 years served as a prototypic model for acute T leukemia, displayed 10-times lower density of Kv and KCa currents compared to previously underexplored CEM and MOLT cell lines. As for KCa density in CEM and MOLT cells, it was comparable to that in activated healthy T cells and manifold higher as compared to resting T cells. These differences will be discussed in the context of Ca<sup>2+</sup> signaling during T cells activation and leukemogenesis. Supported by CONACyT, grants CB-238689 (OD), CB-220793 (IP), Fronteras in Ciencia 140 (OD).

73-POS Board 73

**Ca<sup>2+</sup> Permeability of Bacterial Sodium Channel Heterotetramers Can Explain the EEEE Paradox in Voltage-gated Na<sup>+</sup> and Ca<sup>2+</sup> Channels.**

**Zeyu Zheng**, Olena Fedorenko, Stephen Roberts.  
Lancaster University, Lancaster, United Kingdom.

The highly selective permeation of ions through voltage gated Na<sup>+</sup> and Ca<sup>2+</sup> channels can be explained in terms of fixed negative charged in the pore region ( $Q_f$ ). However, the  $Q_f$  associated with bacterial voltage-gated Na<sup>+</sup> channels (bacNavs) is reported to be equivalent to that found in mammalian Ca<sup>2+</sup> selective channels (i.e. a pore region with an “EEEE motif”;  $Q_f = -4$ ). This anomaly could be explained if we consider the conserved aspartate residue located in domain II (D<sup>2p51</sup>) of L-type Ca<sup>2+</sup> channels and defining the pore with an “EEEEED motif” (i.e.  $Q_f = -5$ ). To investigate this hypothesis, we generated concatemers of bacNavs to allow the asymmetrical mutation of the selectivity filter (SF) and enable 1e step changes in the  $Q_f$  value associated with the SF. Western blot analysis, immunofluorescence microscopy and patch clamp were employed to evaluate the functionality of these concatenated bacNavs and test the hypothesis that a  $Q_f$  value of at least -5 is necessary for Ca<sup>2+</sup> permeation. This approach was corroborated with the use of defined mixtures of cDNAs encoding for NaChBac mutants exhibiting different  $Q_f$  values. Preliminary patch clamp investigations suggest that a  $|Q_f| \geq 5$  is necessary for Ca<sup>2+</sup> permeation, consistent with the hypothesis that Ca<sup>2+</sup> permeation in L-type Ca<sup>2+</sup> channels is mediated by a EEEEEED locus.

The work was supported by EPSRC (grant No.\ EP/M015831/1).

**WEDNESDAY, OCTOBER 11****POSTER SESSION II****3:05 PM – 5:05 PM****Lower Level, Sala de Vestigios**

All posters are available for viewing during all poster sessions; however, below are the formal presentations for Wednesday. Presenting authors with odd-numbered poster boards should present from 3:05 PM – 4:05 PM and those with even-numbered poster boards should present from 4:05 PM – 5:05 PM. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

|  |               |                 |
|--|---------------|-----------------|
| <b>Andrade López, Karla Lisette</b>    | <b>2-POS</b>  | <b>Board 2</b>  |
| <b>Baez-Nieto, David</b>               | <b>5-POS</b>  | <b>Board 5</b>  |
| <b>Barabash, Miraslau</b>              | <b>8-POS</b>  | <b>Board 8</b>  |
| <b>Blecher, Galit</b>                  | <b>11-POS</b> | <b>Board 11</b> |
| <b>Byron, Kenneth</b>                  | <b>14-POS</b> | <b>Board 14</b> |
| <b>Chapman, Mark</b>                   | <b>17-POS</b> | <b>Board 17</b> |
| <b>Cuaxospa-Blancas, Miguel</b>        | <b>20-POS</b> | <b>Board 20</b> |
| <b>De la Rosa, Juan</b>                | <b>23-POS</b> | <b>Board 23</b> |
| <b>Farley, Joseph</b>                  | <b>26-POS</b> | <b>Board 26</b> |
| <b>Flores Aldama, Lisandra</b>         | <b>29-POS</b> | <b>Board 29</b> |
| <b>García Delgado, Neivys</b>          | <b>32-POS</b> | <b>Board 32</b> |
| <b>González-Hernández, Irma</b>        | <b>35-POS</b> | <b>Board 35</b> |
| <b>Hendrix, Jelle</b>                  | <b>38-POS</b> | <b>Board 38</b> |
| <b>Hilton, Jacob</b>                   | <b>41-POS</b> | <b>Board 41</b> |
| <b>Lopez-Rodriguez, Angelica Maria</b> | <b>44-POS</b> | <b>Board 44</b> |
| <b>Martinez Rojas, Vladimir</b>        | <b>47-POS</b> | <b>Board 47</b> |
| <b>Montiel-Jaen, María Guadalupe</b>   | <b>50-POS</b> | <b>Board 50</b> |
| <b>Ng, Leo</b>                         | <b>53-POS</b> | <b>Board 53</b> |
| <b>Orta, Gerardo</b>                   | <b>56-POS</b> | <b>Board 56</b> |
| <b>Pierdominici Sottile, Gustavo</b>   | <b>59-POS</b> | <b>Board 59</b> |
| <b>Samanta, Amrita</b>                 | <b>62-POS</b> | <b>Board 62</b> |
| <b>Sanabria, Hugo</b>                  | <b>65-POS</b> | <b>Board 65</b> |
| <b>Silva, Jonathan</b>                 | <b>68-POS</b> | <b>Board 68</b> |
| <b>Van Horn, Wade</b>                  | <b>71-POS</b> | <b>Board 71</b> |

Posters should be set up the morning of October 10 and removed by noon October 13.

**2-POS Board 2****Subcellular localization of Zip3 and ZnT3 Zinc Transporters in Mouse Spermatogenic Cells**

**Karla Lisette Andrade López**, Ignacio López González, Julio César Chávez Zamora, Paulina Torres Rodríguez, Claudia L. Treviño Santa Cruz.  
Biotechnology Institute, Cuernavaca, Mexico.

The physiological relevance of the micronutrient zinc during spermatogenesis has been reported in different mammals. Zinc ions are involved in the spermatogenic cells (SCs) growth and development, chromatin condensation and modulation of oxidative stress in sperm and structural maintenance of seminiferous tubules. Zinc deficiency reduces testosterone production, which is a fundamental hormone for spermatogenesis; increases oxidative stress, seminiferous tubules atrophy and inhibits spermatids differentiation. On the other hand, an excess of zinc has detrimental effects on spermatogenesis, such as a decrease on sperm motility, arrest of SCs and seminiferous tubules atrophy. Therefore, zinc homeostasis must be strictly regulated. To maintain this homeostasis, two different types of zinc transporters are involved, the Zip and ZnT families. The Zip family is composed of fourteen members involve in the entrance of zinc into the cell. Meanwhile, the ZnT family include ten members responsible removing zinc from the cytoplasm. In spite of the zinc physiological relevance in mouse SCs, little is known about the subcellular distribution and the expression of zinc transporters in the different stages of mouse spermatogenesis. In the present report, we immunodetected the presence of Zip3 and ZnT3 transporters in SCs. Our preliminary results suggest that Zip3 transport is mainly located at the plasma membrane of different SCs types. On the contrary, ZnT3 distribution is preferentially express in cytoplasm and intracellular organelles. Consistently with the Zip3 expression in the plasma membrane we observed that extracellular addition of HCO<sub>3</sub> [25 mM], a Zip3 modulator, and ZnSO<sub>4</sub> [100 μM] increased of intracellular zinc concentration in FluoZin-1-loaded SCs. In conclusion, our preliminary results indicate the expression, at least, of Zip3 and ZnT3 zinc transports in mouse SCs.

Acknowledgements: This work was supported by DGAPA/UNAM: IN204914 to ILG, IN203116 to CT.

**5-POS Board 5****The T-Type Calcium Channel Cav3.3 (CACNA1I) and Schizophrenia: A Case Study in What We Can Learn from Human Population Genetics to Understand Structure-Function Relationships in Ion Channels**

**David Baez-Nieto**, Andrew Allen, Ayan Goshal, Lingling Yang, Jen Q. Pan.  
Stanley Center for Psychiatric Research, Broad Institute of MIT & Harvard, Cambridge, MA, USA.

Whole-exome sequencing of a large Swedish cohort of schizophrenia patients and controls has uncovered an enrichment of neuronal genes with disruptive ultra-rare variants (dURVs) that are associated with schizophrenia risk and converge on specific biological pathways. These dURVs have an allelic frequency of  $\sim 1$  in 100,000, suggesting that they are likely de novo variants private to individuals and that may have a deleterious effect on protein function. Therefore, dURVs are potentially valuable tools to interrogate ion channel structure-function relationships in the context of human disease. CaV3.3 is implicated in schizophrenia risk by a large cohort GWAS study. Using the Flp-In/T-REx system we have generated isogenic stable inducible cell lines for every natural occurring variant in CaV3.3 found in the aforementioned Swedish Schizophrenia Cohort. Taking advantage of high-throughput automated patch-clamp, we have characterized more than 60 different variants, of which around 20 are defined as dURVs in this cohort. We have collected steady-state parameters of activation/inactivation, recovery from inactivation, and peak current density for all variants, and we have found so far that dURVs produce stronger functional alterations in the channel properties compared with “common” variants. The parameters most affected by the dURVs were voltage dependent activation and peak current density, and voltage dependent inactivation was largely unaffected by most variants. We have found key dURVs from schizophrenia patients affecting the voltage dependent activation located in the VSD and gating brake, giving us new insights into how CaV3.3 functions, and how its function may be compromised in schizophrenic patients. Such information contributes to understanding the role of CaV3.3 in schizophrenia and may shape our strategies to restore channel function in this context.

**8-POS Board 8****Brownian Dynamics Study of Permeation and Selectivity of NaChBac and Its Mutants**

**Miraslau L. Barabash**<sup>1</sup>, Dmitry G. Luchinsky<sup>1,2</sup>, William Gibby<sup>1</sup>, Carlo Guardiani<sup>3</sup>, Olena A. Fedorenko<sup>4</sup>, Stephen K. Roberts<sup>4</sup>, Peter McClintock<sup>1</sup>.

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We study the permeation and selectivity of NaChBac, a bacterial tetramer channel, using Brownian dynamics (BD) simulations. The molecular structure of the selectivity filter (SF) has been reconstructed in simulations recently [1], and thus our present study links this structure with newly found experimental properties of the channel and its mutants [2].

We use BD to describe ionic motion through the SF, where the injection of ions is implemented via the Grand Canonical Monte-Carlo scheme [3]. We apply the single-ion potentials of mean force (PMFs), which are obtained from the molecular dynamics (MD) simulations [4, 5] thus reflecting the structure of the channel protein. Ion-ion interactions are modelled via screened Coulomb interactions. The model reproduces the electric current and spatial distribution of ions, which are compared with experimental recordings of conduction and selectivity in the NaChBac wild-type channel (LESWAS) and its mutants, LEDWAS, and LDDWAD [2]. The selectivity between Na and K ions is found to arise from different PMFs seen by these ions, with a non-monotonous current-concentration curve when the particle solutions contain both Na and K ions.

We believe the results can find application in physiology, as well as in artificial nanopores and nanoscale fluid filters. The research was partially supported by the Engineering and Physical Sciences Research Council UK (grant No. EP/M015831/1).

1. C. Guardiani et al., *J. Chem. Theory Comput.* 13 (3), pp 1389–1400 (2017)
2. O.A. Fedorenko, S.K. Roberts (in preparation)
3. W. Im et al., *Biophys J.* 79(2): 788–801 (2000)
4. M.A. Wilson, T.H. Nguyen, A. Pohorille, *J. Chem .Phys.* 141, 22D519 (2014)
5. C. Guardiani et al. (in preparation)

**11-POS Board 11****Regulation of K<sub>2P</sub> Potassium Channels by Membrane Cholesterol**

**Galit Blecher**, Noam Zilberberg.

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Potassium leak channels (K<sub>2P</sub>) play a central role in setting the membrane resting potential. Their activity is regulated by physical and chemical effectors such as temperature, pH, mechanical stretch and phosphorylation. Cholesterol is an essential structural component of mammalian cell membranes and it also is a main component of lipid rafts, which are cholesterol/sphingomyeline enriched microdomains in the membrane. It was found that membrane cholesterol regulates the activity of several membrane proteins. In this study, we describe the mechanism by which membrane cholesterol levels affects K<sub>2P</sub> channels activity.

We studied the influence of membrane cholesterol levels on several members of the K<sub>2P</sub> family: human K<sub>2P2.1</sub>, K<sub>2P3.1</sub>, K<sub>2P5.1</sub> and K<sub>2P9.1</sub> channels as well as K<sub>2P0</sub> channels from *Drosophila melanogaster*, all expressed in *Xenopus laevis* oocytes and studied using the two electrode voltage clamp technique (TEVC). Depletion of membrane cholesterol using Methyl- $\beta$ -cyclodextrin (M $\beta$ CD) altered the activity of most tested channels. Application of 1-5mM M $\beta$ CD reduced K<sub>2P2.1</sub> currents by 80% and increased K<sub>2P0</sub> currents 7-fold. Other channels displayed milder responses. In accordance with these results, sphingomyelin hydrolysis by sphingomyelinase had effects similar to those of M $\beta$ CD on K<sub>2P2.1</sub> channels. Unlike most cholesterol sensitive channels, K<sub>2P2.1</sub> channels are not expressed within lipid rafts and are not affected by the elimination of consensus cholesterol binding domains. While mutant K<sub>2P2.1</sub> and K<sub>2P0</sub> channels, that are insensitive to phosphorylation, were not affected by cholesterol depletion, G-protein activity blockade had no effect. We thus conclude that the activity of members of the K<sub>2P</sub> potassium channels is regulated by membrane cholesterol and speculate that K<sub>2P2.1</sub> channel's activity is regulated via alteration of kinase activity.

## 14-POS Board 14

**Regulation of Vascular Kv7 Channels by G<sub>q</sub>- and G<sub>s</sub>- Coupled Receptor Signaling****Kenneth L. Byron**<sup>1</sup>, Leanne L. Cribbs<sup>2</sup>, Lyubov I. Brueggemann<sup>1</sup>.<sup>1</sup>Loyola University Chicago, Maywood, IL, USA, <sup>2</sup>Loyola University Chicago, Maywood, IL, USA.

Kv7 (KCNQ) potassium channels are important regulators of membrane voltage in excitable cells. We previously found that Kv7.4 and Kv7.5  $\alpha$ -subunits assemble to form the predominant functional channels in mesenteric artery myocytes. These vascular Kv7 channels are regulated by G-protein coupled receptors. Arginine-vasopressin (AVP) activates G<sub>q</sub>-coupled V<sub>1a</sub> receptors and suppresses the activity of vascular Kv7 channels in a protein kinase C (PKC) –dependent manner. Conversely, activity of the same vascular Kv7 channels is enhanced upon activation of  $\beta$ -adrenergic G<sub>s</sub>- coupled receptors, in a protein kinase A (PKA)- dependent manner. The responsiveness of vascular smooth muscle Kv7 channel subunits to both G<sub>q</sub>-coupled V<sub>1a</sub> receptor activation and G<sub>s</sub>-coupled  $\beta$ -adrenergic receptor activation follows the order of Kv7.5 > Kv7.4/Kv7.5 >> Kv7.4. We found that G<sub>q</sub>- and G<sub>s</sub>-coupled receptor-mediated regulation of vascular Kv7 channels was associated with phosphorylation of Kv7.5 channel subunits. When G<sub>q</sub>-coupled V<sub>1a</sub> receptors and G<sub>s</sub>-coupled  $\beta$ -adrenergic receptors were activated in sequence, G<sub>s</sub>-coupled regulation dominated over G<sub>q</sub>-coupled regulation of endogenous Kv7.5 channels in A7r5 vascular smooth muscle cells. Activity of all known Kv7 channels critically depends on the presence of a minor membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>). We hypothesize that the opposing effects of PKC activation and PKA activation are due to differential phosphorylation of Kv7.5  $\alpha$ -subunits, which alters the affinity of the channels for PIP<sub>2</sub>. In recent studies we found that activators of PKA opposed the time-dependent loss of Kv7.5 currents in response to treatments that reduce PIP<sub>2</sub> levels. New evidence suggests that specific serine residues present in Kv7.5, but not Kv7.4, and which are phosphorylated following PKA activation, may account for this effect. Overall, we propose that complex regulation of vascular Kv7 channels by G-protein coupled receptors depends on Kv7.5 channel  $\alpha$ -subunit phosphorylation and modulation of their affinity to PIP<sub>2</sub>.

**17-POS Board 17****Identification of Novel Pore Interactions for Selective Nav1.8 Inhibitors**

**Mark L. Chapman**<sup>1</sup>, Matthew D. Fuller<sup>1</sup>, Sonia Santos<sup>1</sup>, Vladimir Yarov-Yarovoy<sup>2</sup>, Phuong T. Nguyen<sup>2</sup>.

<sup>1</sup>Icagen, Durham, NC, USA, <sup>2</sup>UC Davis, Davis, CA, USA.

Nav1.8 voltage gated sodium channels are expressed predominantly in peripheral nociceptors, playing a key role in action potential propagation in these neurons (1-2). Nav1.8 knockout and antisense studies indicate that the channel has a major function in nociceptive processing in preclinical models of pain and histological samples suggest that Nav1.8 is expressed on primary afferent terminals innervating 'painful' human tissues (3-4). Small molecule modulators selective for Nav1.8 from several chemical series have been disclosed including PF-1247324 (IC<sub>50</sub>: 199 nM against human Nav1.8)(5) and A-803467 (IC<sub>50</sub>: 9 nM)(6). Here we describe the *in vitro* biophysical properties of these compounds and identify amino acid residues within the pore domain that are important for compound-channel interaction. While the compounds are broadly similar in terms of selectivity, they can be differentiated by their unique use- and state-dependence of inhibition. PF-1247324 exhibits significant positive use- and state-dependence of block similar to that observed for local anesthetics (LA). In contrast, A-803467 shows negative use and state dependence of block (partial loss of block) that depends on the magnitude and duration of membrane depolarization. These different biophysical profiles of the two chemotypes appear to result from distinct interactions with the channel. Alanine mutations were constructed at two sites important for LA binding (F1710A and Y1717A)(7). In addition, unique residues present in the S6 segments of Domains II and III of Nav1.8 but not TTX-sensitive Nav channels were mutated. We found that the affinity of PF-1247324 was significantly reduced by mutations at the LA binding site as well as Nav1.8 specific residues, whereas A-803467 affinity was reduced most dramatically by mutation of one unique site. The data suggest PF-1247324 and A-803467 interact with distinct yet partially overlapping regions of the pore domain of Nav1.8.

**20-POS      Board 20****New Insights in the Kinetics of the Kir2.1 Inward Rectifier Potassium Channel****Miguel Cuaxospa-Blancas**<sup>1</sup>, Ramón Latorre<sup>2</sup>, Ubaldo García<sup>1</sup>.<sup>1</sup>Centro de Investigación y de Estudios Avanzados del IPN, Mexico City, Mexico, <sup>2</sup>University of Valparaiso, Valparaiso, Chile.

The Kir2.1 inward rectifier potassium channel belongs to the family known as IRK channels which have been considered as the principal players in the mechanism that permits buffering external potassium concentration, setting the resting membrane potential and modulating the action potential wave form. Several reports have shown that the Kir2.1 channel expression regulates specific aspects in the cell-excitability behavior in cardiac and skeletal muscles, and in the forebrain. Exist evidence corresponding to the single-channels kinetics of homomeric Kir2.1 channel, where authors show that the channel goes through various sub-states of conductance, which are so small that they do not represent any statistical difference. Using cell-attached and inside-out patches from HEK293 cells, we provide new insights which allow us to show that Kir2.1 channel not only presents the occurrence of three open-states of subconductance but also this kinetical behavior is often stable and long lasting, with very low probability of visiting the closed-state. Another special feature we could confirm is that Kir2.1 channel shows burst of activity mainly between the two largest states of subconductance and not in the same way between the closed-state and the smallest state of subconductance. Finally, we performed similar experiments in cells previously incubated with 100  $\mu$ M of PIP2 and the occurrence of states of subconductance was almost abolished, which suggests that the affinity of PIP2 to its specific binding-site is directly related with the prevention of the appearance of sublevels of conduction.

**23-POS Board 23****Mechanisms of Modulation of Voltage-gated Ca<sup>2+</sup> Channels by MDIMP**

**Juan A. De la Rosa**, Teresa Mancilla-Percino, Guillermo Avila.  
Cinvestav IPN, Mexico City, Mexico.

Introduction: Recently, two series of isoindolines derived from  $\alpha$ -aminoacids were synthesized (containing either carboxylic or ester groups), and a theoretical analysis (molecular docking studies) gave rise to the notion that these compounds might modulate voltage-gated Ca<sup>2+</sup> channels (VGCCs). More recently, voltage-clamp experiments in cardiac myocytes led to the exciting discovery that an isoindoline ester derived from L-leucine (MDIMP) effectively modulates VGCCs. Objective: Here we investigate whether this effect can be classified as “fast and selective” for each subtype of cardiac VGCC. Methods: Ca<sup>2+</sup> currents were measured under whole-cell patch-clamp conditions in heterologous expression systems. A fast perfusion system was used, and the activity of VGCCs was measured in the absence and presence of extracellular MDIMP (paired experiments). Results: Cav1.2 (L-type channel) was investigated first. 250  $\mu$ M of MDIMP slowly decreased the magnitude of I<sub>CaL</sub>, reaching a maximum effect in ~10 min (90%). Because of its slow nature, this phenomenon was not further studied. We then focused on Cav2.3 (R-type channel). Remarkably, MDIMP suddenly decreased the magnitude of I<sub>CaR</sub> (within 2-90 s) and the recovery phase was equally fast. This effect was more potent 200-ms after depolarization compared with that estimated at the peak of the current (the IC<sub>50</sub> values were: 78  $\mu$ M and 557  $\mu$ M). In fact, a drastic acceleration in the apparent inactivation rate was also observed. Additional data indicate that MDIMP also rapidly inhibits Cav3.1 (T-type channel), with an IC<sub>50</sub> of 132  $\mu$ M. This action, however, did not involve changes in the activation and inactivation kinetics. Nevertheless, in both cases (Cav2.3 and Cav3.1) the steady-state voltage dependence of inactivation was shifted (-42 mV and -13 mV), in the absence of changes in activation curves. Conclusion: These data indicate that the mechanisms by which MDIMP modulates VGCCs are channel-type specific.

**26-POS Board 26****Alpha 7 (A7) Nicotinic Acetylcholine Receptors (Nachrs) Signal as Both Lgics and Gpcrs**

**Joseph Farley**, Jayharsh Panchal, Kristi DeBoeuf, Mohammad F. Islam.  
n/a, Bloomington, USA.

$\alpha 7$  nAChRs are widely distributed throughout the nervous system, playing important roles in learning & memory, and are also implicated in a variety of disease and neurodegenerative processes including schizophrenia, Alzheimer's and Parkinson's disease, inflammation, cancer, and nicotine addiction. A variety of compounds (agonists, antagonists, PAMs) produce functional and numerical upregulation of  $\alpha 7$  Rs in different cells, implicating multiple signaling pathways and mechanisms. Prolonged nicotine exposures also upregulate  $\alpha 7$  nAChRs, which may contribute to nicotine dependence/addiction. Previously, we found ~2-fold functional and numerical upregulation of alpha7 nAChRs in *Xenopus* oocytes following 12 hr of 100  $\mu$ M nicotine and 7 hr washout. This nicotine-upregulation was dependent upon intracellular  $Ca^{2+}$ , being abolished by BAPTA-AM, and involved several  $Ca^{2+}$ -dependent enzymes (e.g., PP2B, PKC). But upregulation was independent of  $Ca^{2+}$  influx, being unaffected by removal of extracellular  $Ca^{2+}$ . Similar to glycine receptor 1 channel, the  $\alpha 7$  nAChR contains a conserved G protein-binding cluster (GPBC) in the M3-M4 loop. Mutation of  $\alpha 7$  nAChR GPBC (RMKR to AAAA), to block interaction of  $G\alpha_q$  and  $G\beta\gamma$  with the GPBC, completely blocked nicotine-upregulation of  $\alpha 7$  nAChRs. Basal receptor expression levels, peak current amplitude, net charge, and kinetics were all unaffected in the mutants. Conversely, 12 hr exposure to a cell-permeable, competitive antagonist of  $\alpha 7$  nAChRs, methyllycaconitine (MLA; 1  $\mu$ M); a calcineurin inhibitor cyclosporine A (CsA) (10  $\mu$ M); or 2 hr exposure to a dynamin inhibitor - Dynasore (80  $\mu$ M), all produced ~2-fold upregulation of both wt and mutant  $\alpha 7$  Rs. Collectively, our results indicate that  $\alpha 7$  nAChRs function as both ionotropic and metabotropic receptors. Moreover, nicotine appears to modulate the upregulation of  $\alpha 7$  nAChRs, through GPCR signaling, via  $Ca^{2+}$ -dependent inhibition of endocytosis of plasma membrane Rs.

**29-POS Board 29****The Evolution of the Fast Inactivation Phase in Calcium-selective TRP Channels**

**Lisandra Flores Aldama**<sup>1,2</sup>, Daniel Bustos<sup>3</sup>, Juan Opazo<sup>1</sup>, Sebastian Brauchi<sup>1,2</sup>.

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TRPV5 and TRPV6 are highly calcium-selective channels belonging to the Transient Receptor Potential (TRP) family. Both channels are considered as key elements in transcellular calcium transport, therefore, essential in the regulation of systemic calcium homeostasis. It was reported that intracellular calcium ( $\text{Ca}^{2+}$ ) exerts a negative control over the activity of these channels. In this context, the observed channel inactivation has been divided in a fast and a slow phase. The latter depends on the binding of  $\text{Ca}^{2+}$ -Calmodulin complex to the C-terminal of the channel, and it is common between both channels. In contrast, the fast inactivation phase depends on  $\text{Ca}^{2+}$  ions alone and allows to differentiate both channels from a functional point of view. While TRPV6 shows a faster calcium-dependent inactivation, TRPV5 barely inactivates. It has been described that the intracellular loop S2-S3 and residues downstream the transmembrane segment S6 are involved in the differences seen in the kinetics of this fast inactivation, and therefore assumed part of this mechanism. Currently, the exact location of the putative calcium binding site and the molecular mechanism governing this process are not known. The present work propose a structural-functional model for this process. By means of phylogenetic reconstructions, molecular dynamics simulations, site-directed mutagenesis, and patch clamp electrophysiology, we have identified a binding site that put together three different portions of the folded channel, and show that subtle evolutionary-related variations within the binding region account for the differences seen in the fast inactivation phase.

**32-POS Board 32****Age-related Activity and Expression of Voltage-dependent Calcium Channels during Postnatal Development of Pancreatic Beta Cells of Rats**

**Neivys García Delgado**, Myrian Velasco Torres, Carmen Sánchez Soto, Marcia Hiriart Urdanivia.

Instituto de Fisiología Celular, UNAM, Ciudad de México, Mexico.

Pancreatic beta cell acquires functional maturity during the first month of life, allowing them to secrete robust insulin secretion in response to increases in extracellular glucose concentration. Near the ablactating period (20 days) we have observed a period of physiological insulin resistance. We are interested in possible changes in ion channel activity during maturing stages. Within the voltage-dependent calcium channels in beta cells, low-voltage-activated (LVA) have been poorly studied. However, it is well accepted that high-voltage-activated (HVA), specifically type L channels are responsible for the increase of intracellular calcium concentration and insulin exocytosis.

In this work, we analyzed the changes in the expression and activity of calcium channels during the postnatal development of the rat pancreatic beta cell.

Beta cells were obtained from pancreas of neonatal, 20 days and two months male old Wistar rats. Electrophysiological recordings were performed using whole-cell voltage clamp technique. Channels expression was visualized by immunofluorescence.

The detection percentage of LVA current increased with the age of the animal. Peak currents of LVA and HVA channels were close to -10 mV and +20 mV, respectively. Maximum amplitude of both currents were higher in the adult cells. Immunocytochemistry revealed the presence of  $\alpha 1G$ ,  $\alpha 1H$  and  $\alpha 1I$  subunits of LVA channels at all ages. The Cav 3.1 subunit was most expressed. Adult beta cells expressed more Cav 3.1 and Cav 3.2 than 20 days cells.

Together these results show that calcium channels have a differential expression and activity during rat pancreatic beta cell maturation, which is related to insulin secretion.

This work was supported partially by DGAPA PAPIIT-UNAM Grant IN210817, CONACyT Grant CB-253222 and C3 DGAPA PAPIIT Grant IV100116.

**35-POS      Board 35****Structural and Functional Relations of the Voltage-dependent Gated CIC-2 Chloride Channel Assessed by Voltage Clamp Fluorometry.**

**Irma L. González-Hernández**, Roberto Gastélum-Garibaldi, Marina S. Rodríguez-Rangel, Denise M. Alonso-Vázquez, Jorge E. Sánchez-Rodríguez.  
Universidad de Guadalajara, Guadalajara, Mexico.

Voltage clamp fluorometry (VCF) is a powerful technique that provides simultaneous information on the function and conformation changes of an ion channel protein with a high temporal resolution. Here, we used VCF to gain further insights into conformational rearrangements that the voltage-dependent gated CIC-2 chloride (Cl<sup>-</sup>) channel experiments during its function. To this end, we engineered single cysteine mutants on the external facing of the P and Q  $\alpha$ -helices of the mouse CIC-2 chloride channel as targets of a cysteine-reactive fluorescent reporter (TMR). Wild type CIC-2 channel and all cysteine mutants were expressed in *Xenopus laevis* oocytes. Its functional integrity, pH and Cl<sup>-</sup> concentration dependence were assessed by using the cut-open oocyte voltage clamp technique. In agreement with the homodimeric architecture CIC chloride channels, the ionic currents produced by the CIC-2 channels displayed a well-defined time dependence of the fast gating and slow/common gating. Importantly, we have identified the position Q535C (top Q helix) that produce voltage dependent fluorescence changes during its opening and closing gating. These fluorescence intensity changes from Q535C were correlated with its respective open probability curve following a Boltzmann distribution. This finding begins to define an external region of the CIC-2 that is moving during its opening and closing transitions. Suported by PROSNI-UDG/2017 and PRODEP-NPTC 236463 to JESR.

**38-POS Board 38****Unravelling the Structure-function Relationship of the GlyR**

Veerle Lemmens<sup>1,2</sup>, Waldemar Schrimpf<sup>3</sup>, Bert Brône<sup>1</sup>, Jochen Meier<sup>4</sup>, Don Lamb<sup>3</sup>, Johan Hofkens<sup>2</sup>, **Jelle Hendrix**<sup>1,2</sup>

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The glycine receptor (GlyR) is a protein involved in neuron communication. Upon binding of glycine it transports chloride ions, thereby fine-tuning neuron activity. Dysfunction of the GlyR is linked to neurological disorders including hyperekplexia, autism and temporal lobe epilepsy. Furthermore, the alpha 3 type GlyR is a promising target to treat pain, but more fundamental insights on receptor structure and function are needed. As it consists of five subunits, different combinations of subunits lead to receptors with different properties. The diversity between GlyR isoforms is further increased by alternative splicing and RNA editing.

We want to characterize the different  $\alpha 3$  GlyR splice variants (K/L) and investigate the precise mechanism of GlyR channel gating. To determine the GlyR  $\alpha 3$ K/L pentamer composition at the ensemble level in live cells, we recently developed a new fluorescence fluctuation imaging method ('raster spectral image correlation spectroscopy', RSICS). To corroborate the pentamer composition we employ single-molecule sensitive fluorescence imaging. To provide insights into the dynamic channel structure, we employ single-molecule Förster resonance energy transfer, by labeling the receptor on cells using amber suppression technology.

Our final goal is to combine fluorescence microscopy and electrophysiology measurements on a single setup, to link GlyR activity directly with structural properties (e.g. cellular distribution, quaternary composition and overall structure) in real-time and at the single-cell or even single-receptor level. We hope that information from our research can be translated into the development of isoform-specific ligands.

41-POS Board 41

**Probing the Molecular Details of TRPM8 Ligand Gating****Jacob K. Hilton**<sup>1,2,3</sup>, Parthasarathi Rath<sup>1,2,3</sup>, Melanie L. Aprahamian<sup>4</sup>, Steffen Lindert<sup>4</sup>, Wade D. Van Horn<sup>1,2,3</sup>.<sup>1</sup>Arizona State University, Tempe, AZ, USA, <sup>2</sup>Biodesign Institute, Tempe, AZ, USA, <sup>3</sup>Arizona State University, Tempe, AZ, USA, <sup>4</sup>Ohio State University, Columbus, OH, USA.

The human TRPM8 ion channel is a polymodally gated ion channel that is activated by diverse stimuli, including cold temperature, small molecules such as menthol, phosphatidylinositol 4,5-bisphosphate, and regulatory subunit proteins. This channel has garnered increasing interest in recent years due to emerging discoveries of its physiological roles in pain, metabolism and temperature regulation, migraines, and cancer. This makes TRPM8 a potentially attractive therapeutic target; however, the molecular level details of ligand binding and activation are still under investigation. Previous work in our lab has shown that the TRPM8 agonist menthol binds to the S1-S4 ligand-sensing domain. Nuclear magnetic resonance (NMR) and microscale thermophoresis (MST) binding data show that Y745H and R842H mutants, which previous electrophysiology studies had implicated in menthol binding, retain the ability to directly bind menthol with similar affinity as the WT domain. To follow up on these studies, we use a combination of computational modeling techniques and whole-cell patch-clamp electrophysiology to probe channel-ligand interactions. A Rosetta membrane model of the transmembrane domain of human TRPM8 was constructed based on homology to cryo-EM structures of the TRPV1 ion channel, and TRPM8 agonist docking experiments were performed to identify potential binding sites. These results were used to guide the design of TRPM8 mutants which were functionally tested for menthol sensitivity in electrophysiology experiments and eventually subjected to direct binding studies by NMR.

## 44-POS Board 44

**Virtual Rescuing of CNG<sub>F547L</sub> Mutant Channel by Cyclic Nucleotide Analogues**

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Fac. C. Quimicas UJED, Durango, Durango, Mexico.

Cyclic nucleotide-gated (CNG) channels are critical to the photo transduction cascade that underlie in vertebrate vision. These channels open and close in response to light-induced changes in the intracellular cyclic GMP concentration. Approximately, 75% of the mutations related to achromatopsia, a retinal disorder with impaired color vision, are in the genes encoding CNG channels. The F547L mutation is located at the cyclic nucleotide binding domain (CNBD) in the CNG A3 subunit. This mutant channel reaches the plasma membrane, but it is not functional at 37°C. Interestingly, its function is rescued at 27°C or by co-expressing CNG β subunits; nevertheless, apparent affinity to cGMP is increased, suggesting that correct folding of the protein can be induced leading to the functional rescue of the channel.

We have modelled the human CNGA3 channel and the CNGA3<sub>F547L</sub> mutant using as template the crystal structure of the *C. elegans* CNG channel. The model of the mutant suggests a constriction of the structure due to formation of new hydrogen bonds. By docking, we also tested the affinity of natural CNG channel ligands such as cGMP and cAMP along with twelve cyclic nucleotide analogues. Supporting the increased apparent affinity for ligand, previously reported, CNGA3<sub>F547L</sub> mutation induces the reduction in size and volume of CNBD, increasing the score affinity for most of the ligands tested, except two molecules that we consider good candidates to rescue the mutant channel function as they keep the same score affinity in the wild type and mutant channel.

47-POS Board 47

**Functional Coupling between NHE1 Exchanger and TRPA1 Channel in Nociceptive Neurons.****Vladimir A. Martinez Rojas**<sup>1</sup>, Norma L. Gomez Viquez<sup>1</sup>, Francisco Mercado<sup>2</sup>, Janet Murbartian<sup>1</sup>.<sup>1</sup>Cinvestav, Mexico, Tlalpan, Mexico, <sup>2</sup>Instituto Nacional de Psiquiatria Ramon de la Fuente Muñiz, Mexico, Tlalpna, Mexico.

The regulation of the intracellular pH (pHi) in neurons is an elemental physiological process. Changes in pHi affect the functions of enzymes, ion channels, and other macromolecules, thus affecting synaptic transmission and neuronal excitability. It has been reported that inhibition of the Na<sup>+</sup>/H<sup>+</sup> (NHE1) exchanger increases discharge activity in nociceptors and intensify formalin-induced acute and chronic nociceptive behaviors. Besides, TRPA1 receptor is activated by diverse stimulus, among then, alkaline or acid pHi. Since these proteins are expressed in primary afferent nociceptive neurons, TRPA1 channel activity could be closely modulated by NHE1 and contributes to modulate the nociceptive transmission. To test this possibility, we evaluated the responses of NHE1 and TRPA1 with patch clamp recording and microfluorometry in native adult rat dorsal root ganglion (DRG) dissociated neurons.

We first examined changes of the cytosolic pH in BCECF-loaded neurons upon inhibition of NHE1 exchanger with zoniporide. The application of zoniporide (10, 30 and 100 μM) in the small-diameter DRG neurons dissociated, increases the intracellular concentration of protons in dose-dependent manner. In whole-cell patch clamp recording, we found that application of zoniporide no evoke any change on membrane currents in DRG neurons. Therefore, we evocated TRPA1 current through use of 300 μM AITC (specific agonist) and determinate the effect of the inhibition of NHE1 by zoniporide on AITC-induced current. The inward current induced by AITC in DRG neurons underwent a desensitization giving a smaller response on repeated applications. Nevertheless, the grade of desensitization is decreased with the application of zoniporide (30 μM). We conclude that NHE1 and TRPA1 are sensors for intracellular acidification in DRGs and suggest that it functions within the pain pathway to mediate sensitivity to intracellular acidosis.

**50-POS Board 50****Modulation of Store-operated Calcium Channels by a Bis-boronate Ester Derived from L-leucine.**

**María Guadalupe Montiel-Jaen**, Teresa Mancilla-Percino, Guillermo Avila.  
CINVESTAV-IPN, Mexico City, Mexico.

**INTRODUCTION:** Orai1 is a  $\text{Ca}^{2+}$  channel of the plasma membrane that is activated during a process known as store-operated calcium entry (SOCE). A decrease in the sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$  content results in oligomerization of an SR protein (STIM), which promotes the formation of a complex between STIM and Orai1 and ultimately activates the  $\text{Ca}^{2+}$  permeability of the latter. Orai1 can be blocked by  $\text{La}^{3+}$ , and it is also well known that this channel is modulated by certain boron compounds (borinates), such as 2-aminoethyl diphenylborinate or 2-APB. **OBJECTIVE:** Recently, we synthesized a new series of borinates, derived from  $\alpha$ -amino acids, and here we decided to investigate whether one of these novel compounds (bis-boronate ester derived from L-leucine) also modulates Orai1. **METHODS AND RESULTS:** The experimental model was myotubes obtained from the C2C12 cell line, in which we recorded an inward current identical to an  $I_{\text{skCRAC}}$  reported previously in mice myotubes (i.e. with similar biophysical and pharmacological properties; Biophys J 103: 202-11). The store was depleted using the combination of a repetitive stimulation protocol and a  $\text{Ca}^{2+}$  chelator (BAPTA, 20 mM). Under whole-cell patch-clamp conditions, it was possible to record an inward  $\text{Ca}^{2+}$  current that was sensitive to both  $\text{La}^{3+}$  (10  $\mu\text{M}$ ) and 2-APB (10 and 75  $\mu\text{M}$ ). Remarkably, an extracellular application of bis-boronate also modulated this current. More precisely, within 1-2 min of exposure, a relatively fast inhibitory effect was observed, with an  $\text{IC}_{50}$  of 228 nM. This value was approximately 20-fold lower than that observed with 2-APB (4  $\mu\text{M}$ ). Thus, this novel compound acted more potently than a classic modulator of Orai1. **CONCLUSIONS:** The bis-borinates ester derived from  $\alpha$ -amino acids represent novel tools that might help to investigate structural and pathophysiological aspects of Orai1.

**53-POS      Board 53****Mutations in the Polycystin Fold Cause Loss-of-function in PKD Channels**

**Leo Ng**, Thuy Vien, Paul DeCaen.  
Northwestern University, Chicago, IL, USA.

The polycystins (PKD2 and PKD2-L1) is a class of TRP (Transient Receptor Potential) ion channels whose biophysics and function in cell biology are poorly understood. Nonetheless, several mutations in PKD2 have been determined to be causative of Autosomal dominant polycystic kidney disease (ADPKD), a common monogenetic disorder characterized by proliferative cysts which leads to renal failure in adulthood. In a collaboration, we have solved the structure of the homo-tetrameric PKD2 channel using cryo-EM. We will present electrophysiological data in conjunction with the PKD2 structure to provide insights on the specific motifs involved in gating. We drew connections with a homologous channel (PKD2-L1) and identified residues that are involved in voltage sensing and channel modulation. Emphasis was placed on the extracellular “polycystin domain” which is unique to these channels and is a hotspot for ADPKD pathogenic mutations. We also looked at co-localization of these mutants in cilia using superresolution microscopy.

56-POS Board 56

**CatSper Channels Are Activated by PKA in Mouse Sperm****Gerardo Orta**<sup>1</sup>, José L. De la Vega-Beltran<sup>1</sup>, David Martín-Hidalgo<sup>2</sup>, Pablo E. Visconti<sup>2</sup>, Alberto Darszon<sup>1</sup>.<sup>1</sup>Instituto de Biotecnología-Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico, <sup>2</sup>University of Massachusetts, Amherst, MA, USA.

In mammals, sperm become motile during ejaculation and swim up the female reproductive tract where they undergo physiological changes named capacitation before being capable of fertilizing the egg. One of the first signaling events is an intracellular cAMP elevation triggered by HCO<sub>3</sub><sup>-</sup> influx which activates the soluble adenylyl cyclase (sAC)<sup>1</sup>, leading to PKA-dependent protein phosphorylation<sup>2</sup>. CatSper, a sperm exclusive, alkali- and voltage-gated Ca<sup>2+</sup> channel, has been proposed to be responsible for the Ca<sup>2+</sup> influx that accompanies and is necessary for capacitation. The key role of cAMP in capacitation and fertilization was demonstrated using pharmacological tools for gain and loss of function of PKA-dependent pathways<sup>3</sup> and confirmed with sAC null mouse models whose sperm are sterile<sup>4</sup>. We hypothesized that CatSper is the molecular candidate in this mechanistic pathway. We tested this possibility using western blot analysis, Em and [Ca<sup>2+</sup>]<sub>i</sub> measurements, and CatSper whole-cell patch-clamp current recordings. Our results show a direct regulation of CatSper by PKA when stimulated by HCO<sub>3</sub><sup>-</sup> influx. Notably, in whole-cell patch-clamp recordings, cAMP in the pipette solution activates CatSper currents strongly and this effect is blocked by PKA inhibitors H89 and PKI. Pharmacological inhibition of the PKA-pathway and CatSper blockade annul the effect of HCO<sub>3</sub><sup>-</sup> on: [Ca<sup>2+</sup>]<sub>i</sub> levels, Em and CatSper currents. Additionally, our experiments with sperm from CatSper KO mice confirm that HCO<sub>3</sub><sup>-</sup> does not alter the ionic currents obtained under CatSper channel recording conditions. All together our results indicate that CatSper channels are activated by PKA directly, having a major role in the Ca<sup>2+</sup> influx occurring during capacitation. These findings have significant implications for our understanding of fertilization. Support acknowledged from CONACyT Fronteras 71, PAPIIT/UNAM IN205516 and NIH RO1 HD038082-13 to Darszon A. NIH RO1 HD038082-13 to Visconti PE.

59-POS Board 59

**The Dynamic Behavior of the P2X4 Ion Channel in the Closed Conformation**

**Gustavo Pierdominici Sottile**<sup>2</sup>, Agustin Ormazabal<sup>2</sup>, Luciano Moffatt<sup>3</sup>, Juliana Palma<sup>2</sup>.  
<sup>1</sup>n/a, Bernal, Florida, Argentina, <sup>2</sup>Univesidad de Quilmes, Bernal, Argentina, <sup>3</sup>Universidad de Buenos Aires, Buenos Aires, Argentina.

P2X receptors are a family of cationic channels whose opening is triggered by the binding of ATP to the extracellular domain. They are widespread in the tissues of mammals and have a broad range of functions. Due to this fact, an extensive number of investigations were led to explain how they work. In spite of these efforts, many aspects of its functioning remain unclear. One of them corresponds to understand how the closed-to-open transition takes place at a molecular level. Since both (closed and open) conformations of P2X4 have been disclosed when the crystallographic structures were obtained, this constitutes an ideal framework to perform molecular dynamic simulations aimed to get insight into the movements of the system related to the transition.

We will present the results of a detailed molecular dynamics study of the closed form of the P2X4 receptor. The movement of the system were decomposed into inter-chain motions and intra-chain deformations and were compared with the changes that occur in the transition from the closed to the open structure. The analysis revealed that the expansion of the transmembrane helices mainly results from inter-chain motions that already take place in the closed conformation. However, they cannot reach the required amplitude because they are impeded by interactions occurring around the ATP binding pocket. This suggests that the binding of ATP would produce distortions in the chains that eliminate the restrictions on the inter-chain displacements, leading to the opening of the pore. This knowledge not only could contribute to learn about the general mechanisms of how these channels function but also could eventually facilitate the development of potent inhibitors.

62-POS Board 62

**Structural Insight into the Molecular Mechanism of TRPA1 Activation and Inhibition****Amrita Samanta**<sup>1,2</sup>, Janna Kiselar<sup>3</sup>, Seungil Han<sup>4</sup>, Vera Moiseenkova Bell<sup>1,2</sup>.<sup>1</sup>Case Western Reserve University, Cleveland, OH, USA, <sup>2</sup>Case Western Reserve University, Cleveland, OH, USA, <sup>3</sup>Case Western Reserve University, Cleveland, OH, USA, <sup>4</sup>Pfizer Inc., Groton, CT, USA.

Pain, while serving the beneficial function of provoking our attention to dangerous situations, is an unpleasant sensory and emotional experience. Transient Receptor Potential Ankyrin 1 (TRPA1) is a member of the transient receptor potential (TRP) cation channel family and is localized in nerve fibers called “nociceptors” where it plays a key role in the transduction of chemical, inflammatory and neuropathic pain signals from the periphery to the brain.

TRPA1 is a Ca<sup>2+</sup> permeable, nonselective cation channel that is activated by a large variety of structurally unrelated chemical compounds. This diverse group of ligands can be subdivided into two classes: electrophilic and non-electrophilic. Covalent modification of critical cysteine residues on the N-terminus of the channel and/or disulfide bond formation between them is the accepted molecular mechanism of TRPA1 channel opening upon electrophilic activation.

Activation is clearly dependent on a thiol-reactive moiety, thus explaining the structural diversity of this group. Non-electrophilic ligands do not require interaction with critical cysteines of the channel. Although near-atomic resolution structures of TRPA1 were resolved recently by cryo-EM, both in the presence of agonist and antagonists, detailed mechanisms of channel activation and inhibition by these modulators could not be determined.

To determine the structural details of TRPA1 conformation upon agonist and antagonist binding, we used limited proteolysis and mass spectrometry. Our study indicates that conformational rearrangements of the N-terminus ankyrin repeats and the linker region between the cytosolic domain and the transmembrane domain contribute to channel modulation.

**65-POS      Board 65****Submillisecond Dynamics and Activation State of the Ligand-binding Domain of the NMDA Receptor****Hugo Sanabria.**

n/a, Clemson, USA.

N-methyl-D-aspartate (NMDA) receptors belong to the ionotropic glutamate receptor family. However, in comparison to other glutamic receptors, channel gating in NMDA receptors is regulated by the binding of glutamate to the glutamate-binding domain at the GluN2A subunit and glycine to the glycine-binding domain of the GluN1 subunit. Of particular interest, we are set to study the partial agonism of the GluN1 ligand binding domain (LBD). Numerous glutamate receptors have revealed that the LBD closure mechanism relates to the level of agonism, where a full agonist closes the bilobed clamshell cleft of the LBD, whereas a full antagonist opens fully the cleft. Using smFRET and multiparameter fluorescence detection, we revealed the existence of a new conformational state, most likely responsible to initiate channel activation. Two additional conformations are identified, where both are consistent with crystallographic structures. Moreover, we probe the submillisecond dynamics by time-window analysis and we monitor the dynamic equilibrium between the identified conformational states.

**68-POS Board 68****Regulation of Class Ib anti-Arrhythmic Drug Block by the Cardiac Na<sup>+</sup> Channel Voltage-Sensing Domains**

Wandi Zhu, **Jonathan R. Silva**.

Washington University in St. Louis, St. Louis, MO, USA.

**Background:** Clinical studies have shown that class I anti-arrhythmics are helpful in a subset of patients while being lethal in others. For example, patients with Long QT mutations respond differently to mexiletine, a class Ib anti-arrhythmic. We observed the conformational dynamics of different cardiac Na<sup>+</sup> channel (NaV1.5) domains to discover characteristics that facilitate or prevent effective mexiletine block.

**Methods:** NaV1.5 contains four domains (DI-DIV), each with a voltage-sensing domain (VSD). We previously created four DNA constructs that contain a cysteine within a single VSD. Channels were expressed in *Xenopus* oocytes and cysteines were labeled with fluorophores. Ionic current and VSD-tracking fluorescence emission were simultaneously recorded.

**Results:** Mexiletine binding to WT channels stabilizes the DIII-VSD in the activated conformation without affecting the other domains. LQT3 mutant channels show variable mexiletine sensitivity (R1626P>P1332L>WT=S941N>M1652R). These mutants also show varying DIII-VSD activation voltage-dependence, despite the distal locations of the mutations. The DIII-VSD activation shift strongly correlates with mexiletine sensitivity (QT-shortening). The highly-sensitive mutations stabilize the activated DIII-VSD, while the insensitive mutation destabilizes it. Thus, an activated DIII-VSD facilitates mexiletine blockade. To test this hypothesis, we assessed 13 additional mutations, and quantified gating parameters such as conductance voltage-dependence, inactivation, DIII-VSD activation and drug block. A partial least square regression (PLSR) model showed that DIII-VSD activation and inactivation represent components that regulate drug block.

**Conclusion:** Traditionally, channel activation and inactivation were linked to Class-Ib drug block. We propose a novel mechanism where DIII-VSD conformation facilitates or impairs block. By assessing how mutations affect the DIII-VSD, we expect to predict whether a patient will respond to mexiletine. This mechanism could also be utilized to develop a new type of combination therapy by stabilizing the DIII-VSD activated state to increase the efficacy of class-Ib drugs.

**71-POS      Board 71****Probing the Role of the S1-S4 Ligand-sensing Domain in TRPV1 Gating**

Minjoo Kim, Nicholas J. Sisco, Jacob K. Hilton, **Wade D. Van Horn**.  
Arizona State University, Tempe, AZ, USA.

The human TRPV1 ion channel is involved in diverse physiological processes and has been implicated in a variety of pathophysiologicals. Presumably to fulfill disparate functional roles, the channel is modulated by various types of stimuli, including temperature, chemical ligands, pH, voltage, and regulatory proteins. A number of previous studies have identified the S1-S4 membrane ligand-sensing domain as central to vanilloid chemical-based TRPV1 agonism. In an effort to experimentally identify the binding site at the molecular level, the isolated human TRPV1 ligand-sensing domain (hV1-SD) was expressed and purified. NMR characterization of this domain has led to resonance assignment which allows for binding studies that precisely identify the specific residues and atoms key to vanilloid binding. A comparative binding study of the hV1-SD with the canonical vanilloid, capsaicin will be discussed. Additionally, the role of this domain in phosphoinositide lipid binding and its structural and thermodynamic response to changes in temperature will also be highlighted.

**THURSDAY, OCTOBER 12**  
**POSTER SESSION III**  
**3:05 PM – 5:05 PM**  
**Lower Level, Sala de Vestigios**

All posters are available for viewing during all poster sessions; however, below are the formal presentations for Thursday. Presenting authors with odd-numbered poster boards should present from 3:05 PM – 4:05 PM and those with even-numbered poster boards should present from 4:05 PM – 5:05 PM. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

|                                    |               |                 |
|------------------------------------|---------------|-----------------|
| <b>Arazi, Eden</b>                 | <b>3-POS</b>  | <b>Board 3</b>  |
| <b>Baltazar, Enoch</b>             | <b>6-POS</b>  | <b>Board 6</b>  |
| <b>Bendahhou, Saïd</b>             | <b>9-POS</b>  | <b>Board 9</b>  |
| <b>Bondarenko, Olga</b>            | <b>12-POS</b> | <b>Board 12</b> |
| <b>Caba, Laura</b>                 | <b>15-POS</b> | <b>Board 15</b> |
| <b>Chavez-Colorado, Eduardo</b>    | <b>18-POS</b> | <b>Board 18</b> |
| <b>Dämgen, Marc</b>                | <b>21-POS</b> | <b>Board 21</b> |
| <b>Droguett, Karla</b>             | <b>24-POS</b> | <b>Board 24</b> |
| <b>Farley, Joseph</b>              | <b>27-POS</b> | <b>Board 27</b> |
| <b>Flynn, Galen</b>                | <b>30-POS</b> | <b>Board 30</b> |
| <b>Gomez-Laquenas, Froylan</b>     | <b>33-POS</b> | <b>Board 33</b> |
| <b>Green, Michael</b>              | <b>36-POS</b> | <b>Board 36</b> |
| <b>Hernández, Ricardo</b>          | <b>39-POS</b> | <b>Board 39</b> |
| <b>Islas, Angel</b>                | <b>42-POS</b> | <b>Board 42</b> |
| <b>Loyo Celis, Verónica</b>        | <b>45-POS</b> | <b>Board 45</b> |
| <b>Mironova, Galina</b>            | <b>48-POS</b> | <b>Board 48</b> |
| <b>Musgaard, Maria</b>             | <b>51-POS</b> | <b>Board 51</b> |
| <b>Ocelotl-Ovied, Jose</b>         | <b>54-POS</b> | <b>Board 54</b> |
| <b>Ozturk, Tugba</b>               | <b>57-POS</b> | <b>Board 57</b> |
| <b>Pomès, Régis</b>                | <b>60-POS</b> | <b>Board 60</b> |
| <b>Sampedro Castañeda, Marisol</b> | <b>63-POS</b> | <b>Board 63</b> |
| <b>Sanchez Osuna, Jorge</b>        | <b>66-POS</b> | <b>Board 66</b> |
| <b>Stock, Leticia</b>              | <b>69-POS</b> | <b>Board 69</b> |
| <b>Wu, Jason</b>                   | <b>72-POS</b> | <b>Board 72</b> |

Posters should be set up the morning of October 10 and removed by noon October 13.

**3-POS      Board 3****Monoterpenes are Powerful K2P Channels Regulators**

**Eden Arazi**<sup>1,2</sup>, Noam Zilberberg<sup>1,2</sup>.

<sup>1</sup>Ben-Gurion University Of The Negev, Beer-Sheva, Israel, <sup>2</sup>Zlotowski Center for Neuroscience, Beer-Sheva, Israel.

Two pore domain potassium channels (K2P) display constant conductance in the physiological membrane potential range and their activity is highly regulated by both chemical and physical modulators. K2P channels' activity affect numerous physiological processes such as cardiac function, pain perception, depression, neuroprotection and cancer development. Terpenes are a large family of membrane soluble compounds, mostly produced in plants. Several monoterpenes were found to affect the activity of a variety of ion channels. To date, the effect of terpenes on K2P channels has not been studied.

Here we report that terpenes are powerful K2P channel regulators. Human channels were expressed in *Xenopus* oocytes and currents were measured using the two-electrode voltage clamp technique. Channels' activity was found to be highly affected by monoterpenes. For instance, K2P2.1 and other mechanosensitive K2P channels currents were enhanced several-fold by carvacrol, thymol and menthol and decreased two-fold by eugenol. The effect of monoterpenes was not limited to the known stretch-activated K2P channels, as K2P5.1 and K2P18.1 currents were elevated by up to 15-fold and K2P1.1 currents were decreased 3-fold by application of carvacrol. Furthermore, the influence of terpenes on K2P2.1 currents was found to be: A. mediated by its carboxyl terminal, as its removal abolished their effect and B. indifferent to phosphorylation of a conserved PKA site.

Our results demonstrate, for the first time, that terpenes are powerful modulators of K2P potassium channels, that the nature of the effect is channel and terpene specific and that the mechanism of action involves the C-terminus regulatory domain.

**6-POS Board 6****The Conotoxin Cr1 Potently Inhibits the Oncogenic Potassium Channel Kv10.1**

**Enoch Luis Baltazar**<sup>1</sup>, Arlet Loza<sup>2</sup>, Sergio Róman-González<sup>3</sup>, Roberto Arreguin-Espinosa<sup>3</sup>, Hernández-Cruz Arturo<sup>2</sup>, Picones Arturo<sup>2</sup>

<sup>1</sup> CONACyT - Instituto de Fisiología Celular, <sup>2</sup>Laboratorio Nacional de Canalopatías, Instituto de Fisiología Celular, UNAM; <sup>3</sup>Instituto de Química, UNAM.

In recent years, ion channels have been proposed as potential tumor-promoters as well as therapeutic targets for different types of malignancies. One of most studied ion channels in cancer is the voltage-gated potassium channel ether à go-go 1 (Eag1 or Kv10.1). Different studies have shown that Kv10.1 expression induces proliferation of several cancer cell lines and in vivo tumor models, while blockage or silencing of the channel inhibits proliferation. Due to these findings, Kv10.1 has been proposed as an early cancer biomarker. Moreover, its localization as a transmembrane protein, makes this channel an ideal drug target. The goal of this work is to identify new and potent Kv10.1 channel modulators. Using HEK293 cells stably expressing the human Kv10.1 potassium channel (HEK-Kv10.1), we initially screened 40 different new potential modulators by measuring Tl<sup>+</sup> inflow by a fluorescence-based assay (FLIPR® Potassium Assay Kit). We found that Cr1, a polypeptide toxin isolated from the marine cone snail *Conus regularis* (a worm-hunting species collected along the Pacific coast of Mexico), decreased the Tl<sup>+</sup> influx mediated by Kv10.1 channels. In whole-cell patch-clamp recordings from HEK-Kv10.1, outward K<sup>+</sup> currents were activated from a holding voltage of -70 mV by 1-s duration voltage ramps from -100 to +50 mV applied every 5 seconds. Cr1 (1 nM) inhibits peak current amplitude by 75% measured at +50 mV. Cr1 inhibition showed no voltage dependency. Conductance-voltage relationship fitted with a Boltzmann equation gave that Cr1 shifts the half-activation voltage (V<sub>1/2</sub>) from 23.2 ± 1.6 mV to 57.9 ± 14.1 mV (control versus 1 nM Cr1, respectively), without changing the slope factor. Cr1 exerted a concentration-dependent inhibition of Kv10.1 channels with an IC<sub>50</sub> of 4.5 pM. Cr1 inactivation by heating (75 °C for 30 min) and sonication (10 min) completely abolished its inhibitory effects on Kv10.1 currents, in accordance with its polypeptide nature. Our results demonstrate for the first time, a polypeptide toxin with a potent inhibitory effect on Kv10.1 channel activity, then making Cr1 an attractive compound targeting Kv10.1 with outstanding therapeutic potential. Supported by grants 279820 (Laboratorio Nacional de Canalopatías), CB 240305 (Consejo Nacional de Ciencia y Tecnología; CONACyT), México and Dirección General de Asuntos del Personal Académico (DGAPA-UNAM,) PAPIIT IN211616.

**9-POS      Board 9****Insights into the Role of the Kir2.1 K<sup>+</sup> Channel during Osteoblastogenesis**

Julia Matonti<sup>1</sup>, Jonathan Pini<sup>1</sup>, Serena Giuliano<sup>1</sup>, Dina Simkin<sup>2</sup>, Matthieu Rouleau<sup>1</sup>, **Saïd Bendahhou<sup>1</sup>**.

<sup>1</sup>UMR7370 CNRS - Université Côte d'Azur, Nice, France, <sup>2</sup>Northwestern University, Chicago, IL, USA.

The inwardly rectifying K<sup>+</sup> channel Kir2.1 is widely expressed in excitable and non-excitable tissues, and is encoded by the KCNJ2 gene. It plays a key role in maintaining the resting membrane potential in excitable tissues, and ensures the late repolarization phase in the cardiac action potential. Mutations on the KCNJ2 gene have been associated with Andersen's syndrome, short QT syndrome (SQT3), and atrial fibrillation. Andersen's syndrome (AS) is a rare and complex disorder presenting with skeletal muscle, cardiac muscle, and bone defects. AS-associated heterozygous de novo mutations lead to a loss of Kir2.1-associated inwardly rectifying current due to the dominant-negative nature of the disorder. While the function of the Kir2.1 channel can be anticipated in excitable cells, its role in bone development remains to be elucidated.

We have generated human induced pluripotent stem (iPS) cells from healthy and AS patient muscular biopsies and have shown that this can be a good model for AS disorder. These cells were used to investigate the impact of Kir2.1 loss of function during osteoblast differentiation. We show that loss of Kir2.1 channel function down regulates master gene expression during the osteoblast differentiation, leading to defective osteoblasts. This down regulation is due to a disruption of the Bone Morphogenetic Proteins (BMP) signaling pathway. Our work reveals how the electrogenic activity of Kir2.1 K<sup>+</sup> channels at the cell surface can control intracellular signaling.

## 12-POS Board 12

**The Effect of Arachidonic Acid (AA) on T-type  $\text{Ca}^{2+}$  Currents in Mouse Spermatogenic Cells**

**Olga Bondarenko**, Ignacio López-González, Alberto Darszon.  
Instituto de Biotecnología, UNAM, Cuernavaca, Morelos, Mexico.

Mouse spermatogenic cells express T-type  $\text{Ca}^{2+}$  currents ( $I_{\text{CaT}}$ ), which are encoded by  $\text{Cav}3.1$  and  $\text{Cav}3.2$  genes and could play a relevant role in the spontaneous  $\text{Ca}^{2+}$  oscillations during spermatogenesis. However, the mechanism by which T-type channel currents are regulated during spermatogenesis is still unclear. A previous report documented the presence of the  $\alpha/\beta$  hydrolase domain-containing protein 2 in the male germ line, which can be activated by different hormones and produces arachidonic acid (AA) and glycerol from 2-arachinoylglycerol. In this study we investigated the potential regulation of spermatogenic cell T-type  $\text{Ca}^{2+}$  currents by AA. To this effect we recorded  $I_{\text{CaT}}$  in whole cell electrophysiological recordings in spermatogenic cells in the absence or presence of different AA concentrations. AA inhibits  $I_{\text{CaT}}$  in a dose-dependent manner, with an  $\text{IC}_{50}=186$  nM without shifting the I-V curve.  $I_{\text{CaT}}$  lacks run down in control conditions and AA-induced  $I_{\text{CaT}}$  inhibition was reached 5 minutes after addition and was stable through time. The  $I_{\text{CaT}}$  inhibition by external AA was reverted pre-incubating this compound in presence of albumin (BSA, 1%), suggesting AA incorporates into the spermatogenic cell plasma membrane. Consistently, longer incubation of spermatogenic cells with different AA concentrations reduces the BSA potency to revert the AA-induced  $I_{\text{CaT}}$  inhibition, possibly indicating a higher incorporation of AA into the spermatogenic cell plasma membrane. Finally, preliminary results show AA does not modify the time to peak or the inactivation kinetics of the  $I_{\text{CaT}}$  suggesting a reduction of the available  $\text{Ca}^{2+}$  channel fraction as the inhibitory mechanism of  $I_{\text{CaT}}$ .

Acknowledgements: OB is a fellow from DGAPA-UNAM (Mexico). This work was supported by CONACyT Fronteras 71 to AD; PAPIIT/UNAM: IN205516 to AD, and IN204914 to ILG; NIH RO1 HD038082-13 to AD.

**15-POS Board 15****Minocycline Inhibits ASIC Currents in Dorsal Root Ganglion Neurons by a Selective Action on the ASIC1a Channel Subunit**

**Caba Sánchez Laura Cecilia**<sup>1</sup>, Soto Eguibar Enrique<sup>1</sup>, Vega Y Saenz de Miera Maria del Rosario<sup>1</sup>, Félix Grijalva Ricardo<sup>2</sup>.

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Acid Sensing Ion Channels (ASIC) are proton-activated Na<sup>+</sup> channels expressed in the nervous system, where they are involved in learning, fear behavior, neurodegeneration, nociception, mechanoreception, chemoreception, ischemia, epilepsy, inflammation, among others. Thus, the discovery of pharmacological agents targeting ASICs has high therapeutic potential. Minocycline is a semisynthetic antibiotic of the tetracycline family that has neuroprotective properties, in processes in which ASICs have been involved (such as neurodegeneration, nociception, and inflammation), which led us to postulate the ASICs as a minocycline target. As we hypothesized, whole cell voltage clamp recordings from isolated dorsal root ganglion (DRG) neurons from the rat, shown that minocycline inhibits the peak amplitude of proton gated current (ASIC) neurons in a dose-dependent manner (IC<sub>50</sub> ~ 100 μM). In heterologous expression system CHO-cells, minocycline selectively inhibits the ASIC1a currents without significant inhibitory effects in ASIC2a, ASIC1b or ASIC3 currents. Molecular anchor analysis (in-silico analyses) suggests the “acid pocket” and the “central vestibule” as putative minocycline bindings sites in the chicken ASIC1a channel. These results demonstrate the inhibitory action of minocycline on the ASIC currents, contributing to account for the neuroprotective action of minocycline, and opening a venue for research of tetracycline derived molecules as potentially relevant drugs to manipulate ASIC currents, and to improve neuroprotective therapeutic armamentarium.

**18-POS      Board 18****Dissecting a High-affinity Binding Region for T-type Calcium Channels Antagonists.**

**Eduardo Chavez-Colorado**, Uriel J. Vazquez-Ayala, Zazil Herrera-Carrillo, Juan C. Gomora. Instituto de Fisiología Celular-UNAM, Mexico City, Cd Mx, Mexico.

T-type calcium or Cav3 channels are involved in several crucial cell functions. Remarkably, Cav3 channels are implicated in pathophysiological processes such as epilepsy, sleep disorders, hypertension, neuropathic pain and cancer. However, the lack of potent and selective antagonists of Cav3 channels has been a setback for further investigation of these channels function in neurological diseases and to validate them as efficient drug targets. Recently, a series of potent and selective T-type calcium channel blockers (T-Type Antagonists or TTAs) have been reported in several studies. Here, we have gain insights into the binding site for the molecular coupling of TTA-A2 ([2-(4-cyclopropylphenyl)-N-((1R)-1-{5-[(2,2,2-trifluoroethyl)oxo]pyridin-2-yl}ethyl)acetamide]) on the human Cav3.1 T-type calcium channel. By combining molecular simulations, mutagenesis and patch-clamp experiments on Cav3.1 channels expressed in HEK-293 cells, we have collected experimental evidence about the molecular basis of TTA-A2 binding site in this channel. The binding site is mainly outlined by amino acid residues from S6 segments of Domain I and IV. We also confirmed that the binding site is conserved in the three human Cav3 channels, but not in the L-type channels, as suggested by sequence alignments. In addition, we report the existence of a high affinity binding region for other structural and functional diverse TTAs (e.g., mibefradil, TTA-P2, penfluridol, etc.); and furthermore, we provide evidence that TTA-Q4, a positive allosteric modulator of TTA-A2 blocking effect, does not share the same binding site with the latter. Our results contribute to the knowledge for the rational design of T-type channel antagonists based on the structure of the TTA-A2 binding site, and might represent a breakthrough for understanding the kind of physical interaction that allow these compounds to modulate differentially the T-type calcium channels subfamily.

Supported by CONACYT-MEXICO 167790 and PAPIIT-UNAM IN207614.

**21-POS      Board 21****Studying Protein-lipid Interactions of a Pentameric Ligand-gated Ion Channel in a Synaptic Membrane as the Basis for Targeting Allosteric Modulation Sites for Drug Design**

**Marc A. Dämgen**, Philip C. Biggin.  
University of Oxford, Oxford, United Kingdom.

Pentameric ligand-gated ion channels (pLGICs) mediate fast synaptic transmission and are involved in a range of neurological disorders such as Alzheimer's, Parkinson's or epilepsy which makes them pivotal drug targets. However, existing drugs suffer from side effects due to poor subtype selectivity. Targeting allosteric modulator sites has great potential to overcome this problem for the compelling reason that they exhibit more sequence diversity between receptor subtypes than the highly conserved orthosteric sites. Many allosteric sites are located at the lipid-accessible regions of the transmembrane domain. It has therefore been hypothesised that lipids themselves can act as allosteric modulators by interacting with these sites and thus modifying the free energy landscape between functional states. Hence, it is a prerequisite to the understanding of allosteric modulation to firstly characterise the protein-lipid interactions in the natural membrane environment. Although it is known that certain lipid types such as cholesterol, anionic lipids or sphingolipids are crucial for the function of pLGICs, the exact nature of the interactions and how they modulate the receptor remains poorly understood. To shed light into these questions, for the first time we have simulated a pLGIC embedded in a realistic synaptic model membrane containing all lipid types that are crucial for its function. We study the glycine receptor as a representative of the pLGIC class since high quality structures in key functional states are available. Using coarse-grained simulations we can access time scales to obtain a converged lipid distribution, allowing us to characterise the annular lipid shell. Additionally, with all-atom simulations we draw a detailed picture of atomistic protein-lipid interactions with a special focus on allosteric sites, thus elucidating the basis for allosteric modulation.

24-POS Board 24

**Hemichannel Closure Triggered by Extracellular ATP at Millimolar Concentration Impairs Airways Ciliary Activity**

**Karla Droguett**, Mariana Rios, Lillian Arzola, Manuel Villalón, Nelson P. Barrera.  
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Mucociliary clearance (MCC) in the airway epithelium protects the lungs against contaminants and microorganisms present in the inspired air. However, in inflammatory chronic respiratory diseases the MCC is impaired, which in turn contributes to airway inflammation and respiratory disease progression. Extracellular ATP is a signalling molecule in the epithelium regulating both basal ciliary beat frequency (CBF) and increased CBF, associated to  $[Ca^{2+}]_i$ , after mechanical stimulation. In addition, the ATP effect on CBF is dependent on pannexin and connexin hemichannels (Panx/Cx HCs) activity, through an autocrine purinergic mechanism involving ATP release and  $Ca^{2+}$  entry. Under pathophysiological conditions of the airways, such as asthma, high levels of ATP had been measured in broncho alveolar fluid, however it is unclear how these ATP concentrations affect ciliary activity and MCC. Our goal was to determine the cellular effect of 1 mM ATP on primary cultures of mouse tracheal epithelium. A series of experimental methods were carried out, hemichannel functionality through the uptake of ethidium bromide (EtBr), CBF and ciliary beating forces using atomic force microscopy, and  $[Ca^{2+}]_i$  measurements by fluorimetric assays. The ATP addition reduced the uptake rate of EtBr, effect that was also observed in the presence of carbenoxolone (100  $\mu$ M), a Panx/Cx HCs inhibitor. The  $Ca^{2+}$  ionophore ionomycin (10  $\mu$ M) increases the uptake rate of EtBr, which was blocked by ATP. Furthermore, ATP lowered CBF, ciliary forces and  $[Ca^{2+}]_i$ . These results show that high levels of extracellular ATP, measured in chronic respiratory diseases, trigger epithelial hemichannel closure that impairs airway ciliary activity and MCC. Funded by CONICYT-FONDECYT 3150652 (KD), CONICYT 21160416 (LIA) and CONICYT-DPI20140080.

27-POS Board 27

**Amyloid Beta Peptide Fragments 1-42 and 25-35 Suppress Kv1.1 Channel Activity****Joseph Farley**, Kristi DeBoeuf, Mohammad F. Islam.

n/a, Bloomington, USA.

Many studies have found that A $\beta$ -peptides participate in the pathogenesis of Alzheimer's disease, leading to disruption of Ca<sup>2+</sup> homeostasis and eventual neurotoxicity. The mechanisms underlying these effects remain unclear. We suggest that A $\beta$ -inhibition of voltage-dependent K<sup>+</sup> channel (e.g., Kv1.1) activity is among the earliest steps. We previously elucidated a pathway in which Ca<sup>2+</sup>-dependent activation of PP2B, PKC, PTKs, and RhoA all contributed to rapid strong suppression of Kv1.1 activity in *Xenopus* oocytes. This pathway is recruited by a variety of stimuli that increase [Ca<sup>2+</sup>]<sub>i</sub>, including GPCRs that couple to Gq/11 –PLC, and Ca<sup>2+</sup> ionophore. Because Kv1-family channels regulate depolarization and Ca<sup>2+</sup> influx, and inhibition of Kv1 channels can be neurotoxic, we speculate that A $\beta$ -suppression of Kv1 channels could lead to hyperexcitability, altered synaptic transmission, disrupted Ca<sup>2+</sup> homeostasis, and neurotoxicity. We assessed the effects of the A $\beta$ (1-42) peptide and the core fragment [A $\beta$ (25-35)] on murine Kv1.1 channels expressed in oocytes. A $\beta$ (1-42) [10 nM -1  $\mu$ M] produced dose-dependent inhibition of Kv1.1 current, ~50% reductions within 30 m for 1  $\mu$ M. A $\beta$  suppression of Kv1.1 was partially Ca<sup>2+</sup>- and PP2B-dependent, being reduced by ~50% when cells were loaded with BAPTA-AM, or exposed to the PP2B-inhibitor cyclosporine A. Patch-clamp results suggest that A $\beta$ -suppression of Kv1.1 involves both PP2B-dephosphorylation and direct protein-protein interaction of A $\beta$  with Kv1.1 channel subunits. Exposure of inside-out single Kv1.1 channels in ripped-off oocyte patches to purified catalytically-active PP2B produced gradual reductions in *p*(open), followed by abrupt disappearance of Kv1.1 activity. Application of A $\beta$  to the intracellular face of Kv1.1 channels also produced dramatic reductions in *p*(open). We also found that 2  $\mu$ M of the toxic core A $\beta$ (25-35) suppressed Kv1.1 currents by ~40%. Using “tip-dip” artificial membranes, 1  $\mu$ M A $\beta$ (25-35) suppressed Kv1.1 channels when applied to the intracellular face.

**30-POS      Board 30****Molecular Mechanism Coupling the S4 Voltage-sensor to the Pore Domain in HCN Channels.**

**Galen E. Flynn**, William N. Zagotta.  
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Hyperpolarization-activated cyclic nucleotide-modulated (HCN) channels are both voltage- and ligand-activated cation channels that functionally contribute to pace-making activity in cardiac and neuronal cells. HCN channels are members of the voltage-gated K<sup>+</sup> channel superfamily. However, HCN channels are unique in that they are activated by hyperpolarizing voltages as well as by the direct binding of cyclic nucleotides. Recently, a cryo-EM 3D structure of the human HCN1 channel revealed that the voltage-sensing domains (VSDs) and pore domains (PDs) of a single subunit are juxtapose in the tetrameric complex and not swapped between subunits as observed for Kv1.2 channels (Lee & MacKinnon, 2017, Long et al., 2005). This arrangement of transmembrane domains begs the questions: 1) how are the VSD and PD electro-mechanically coupled and 2) what is the role of the S4-S5 linker in voltage-dependent activation of HCN channels? To address these questions, site-directed mutagenesis was used to perturb the S4-S5 linker region of spHCN. Excised inside/out patch-clamp techniques were used to record macroscopic currents from spHCN channels heterologously expressed in *Xenopus* oocytes. Conductance-voltage relationships, measured in the absence or presence of saturating concentrations of full agonist cAMP or partial agonist cGMP, were fit with a modified Horrigan and Aldrich (2002) allosteric model. Major findings were: 1) the S4-S5 linker was not required for voltage-dependent activation or cyclic nucleotide-dependent modulation, 2) the S4C-term was required for voltage-dependent activation, 3) the S5N-term was involved in pore opening, and 4) both the S4C-term and the C-terminus acted as auto-inhibitory domains on the pore. These findings provide new insights into the molecular mechanism of voltage-dependent activation in HCN channels.

**33-POS      Board 33****Inhibition of the K<sup>+</sup>-Conductance and Cole-moore Shift of the Tumor Related Kv10.1 Channel by Amiodarone**

Carolina Barriga-Montoya, Areli Huanosta-Gutiérrez, Arturo Reyes-Vaca, Arturo Hernández-Cruz, Arturo Picones, **Froylán Gómez-Lagunas.**

Universidad Nacional Autónoma de México, Ciudad de México, Mexico.

Ectopic overexpression of the voltage-dependent Eag1 (Kv10.1) K<sup>+</sup> channel is associated with the cancerous phenotype of about 70% of human cancers and tumor cell lines. Recent reports show that, compared with the canonical Shaker-related Kv family, Kv10.1 presents unique structural and functional properties. Herein we report the interaction of the class III anti-arrhythmic compound amiodarone with Kv10.1. We found that amiodarone inhibits the K<sup>+</sup>-conductance with nanomolar affinity. Additionally amiodarone inhibits the characteristic Cole-Moore shift of Eag channels. Our observations are interpreted considering the recently reported structure of the channel. We conclude that amiodarone binds both to the pore and voltage sensor modules, including within the latter the cytoplasmic Per-Arnt-Sim-domain which is known to regulate the voltage dependency of Kv10.1.

## 36-POS Board 36

**Quantum Calculations on a Voltage Sensing Domain of Kv1.2: H<sup>+</sup> Transfer and Gating Current**Alisher M. Kariev<sup>1</sup>, **Michael E. Green**<sup>1</sup>.<sup>1</sup>n/a, New York, USA, <sup>2</sup>City College of Cuny, New York, NY, USA.

Quantum calculations on a 904 atom segment of the voltage sensing domain (VSD) of Kv1.2, with 24 water molecules added, starting from X-ray coordinates (pdb:3Lut), show how protons may traverse the section, and become the gating current. With the S4 segment free to move, and a -107 Vm<sup>-1</sup> field applied (equivalent membrane potential:  $V \approx -70$  mV), the segment backbone moves  $< 2.5$  Å, and not in the intracellular direction, as required in standard models of gating. Second, we have optimized (energy minimized) structures with protons in several positions, testing whether salt bridges present in the VSD may be neutralized; earlier work has shown that salt bridges in the absence of water do not ionize (Liao and Green, CompTheoChem, 2011). Here, energy is lower for the unionized form in certain cases with  $V < 0$  (closed channel), but not with  $V \geq 0$  (open channel). Specific cases which would add to gating current, including a transfer of a proton from a tyrosine (Y266) to the pre-guanidinium nitrogen of a neighboring S4 arginine, are shown, with accompanying energy for the proton in each position. For the lowest energy states, closed (-70mV) the charges on Y266, R300, and E183 are 0,0,0; at 0 mV (open), -,+,0, respectively (nominal charges—actual charges are not integral). Open energy is  $\approx 15$ kBT higher. The proton appears able to move to E183 by way of R300, providing in total about 0.6 charges of gating current. Optimization to get structure used HF/6-31G\*, followed by more accurate energy calculation at B3LYP/6-31G\*\*, plus NBO for atomic charges. Additional cases have been calculated, including some with  $V = +70$ mV; the open and closed cases shown above have the lowest energy of a substantial set of plausible states.

**39-POS Board 39****On the Hyperpolarization Activated Ion Current of *Xenopus* Oocytes, Its Relation to HCNs Expression and Gating Properties of HCN2 Mutants**

**Ricardo Hernández**, Adriana Pétriz, Edith A. Espino-Saldaña, Adán Hernández, Ataúlfo Martínez-Torres.

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Frog oocytes present a diversity of ion conductances including one that is activated upon extreme negative hyperpolarizing potentials. In an attempt to identify the molecular component of this current, the four cDNAs that code for the *Xenopus tropicalis* hyperpolarization activated cyclic nucleotide-gated channels (HCN1-4) were cloned, expressed in HEK293 cells and single-site mutants of a conserved series of amino acids neighboring the selectivity filter were generated and studied

The ion currents induced by hyperpolarizing pulses were recorded using two-electrode, voltage-clamp in oocytes of *X. tropicalis*, stepping the membrane from -60 mV to -140. The genome sequence of this frog was explored to determine the presence of HCN-encoding genes, cDNAs were identified, cloned, introduced into expression plasmids, and transfected in HEK293 cells or used as templates to produce mRNA for expression in *X. laevis* oocytes and recorded by voltage-clamp

In *X. tropicalis* oocytes the current activates and inactivates slowly. The current-voltage relationship showed an inward rectification at potentials more negative than -130 mV and the reversal potential was -23 +/- 1.5 mV. Antisense oligonucleotides to HCN1-4 did not block the ion current. The cDNAs of HCN1, 2 and 3 resulted as predicted from GeneBank whereas HCN3 forms a truncated channel predicted to be non-functional and was named HCN-like. HCN-like, co-expressed with the other HCNs, blocked the generation of functional channels. Finally, the HCN2 mutant LCI -> AAA shows changes in the activation and inactivation kinetics, in activation threshold and potassium modulation

In conclusion, we show that HCN RNAs are expressed in *X. tropicalis* oocytes. The HCN-like RNA heteromerizes with other HCNs and blocks their expression. The 391LCI393 amino acid sequence of HCNs is important to provide an effective gating of the ion channel

## 42-POS Board 42

**Mefloquine: a Local Anaesthetic Site Binder with a Unique Structure-activity Profile on the Mammalian Na<sup>+</sup> Channel**

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Mefloquine is a cation channel inhibitor, originally co-developed by the US Department of Defense to treat malaria. By combining site-directed mutagenesis, electrophysiology, molecular modelling, docking and Molecular Dynamics (MD), we were able to predict a loss and gain-of-function of the mefloquine-induced block of rat Nav1.4 channels. This antimalarial may occupy the local anaesthetic binding site (LABS) inasmuch as the mutant Phe1579A increased the IC<sub>50</sub> by two-fold. All-atom MD combined with rigid-protein and induced-fit docking posits that (11S,12R)-mefloquine binds the rNav1.4 channel at a hydrophobic cavity that communicates the LABS with the selectivity filter, which coincides with the latest local anaesthetics binding mode, but differs in that this drug binds above Phe1579, the side of which fluctuates from a proximal to a distal conformation from the channel permeation pathway during the simulations. Although, like most local anaesthetics, mefloquine showed use-dependent increase in block, the current rundown did not increase at escalating frequencies. This compound prominently accelerated peak current decay, with no effect on recovery from inactivation and the co-expression of the  $\beta$ 1 subunit, which significantly accelerates the inactivation and recovery kinetics of most mammalian Na<sup>+</sup> channels, increased the IC<sub>50</sub> of mefloquine from 67 to 128  $\mu$ M. Taken together these results suggest that, in contrast to the hallmark action mechanism of local anaesthetics, mefloquine does not stabilize Na<sup>+</sup> channels in the inactivated state, but it preferentially binds activated channels facilitating inactivation. However the apparent drug affinity to inactivated channels ( $K_i$ ), derived by differences in the steady-state inactivation, suggest drug binding preference to inactivated channels than to channels in the resting state, *i.e.*  $K_i=9.6 \mu$ M, if  $IC_{50} \approx K_r$ , ( $IC_{50}=67 \mu$ M).

45-POS Board 45

**Electrophysiological Characterization of Ionic Current in Sea Urchin Sperm****Verónica Loyo Celis**, Gerardo Orta, Alberto Darszon.

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Sea urchins are widely used in studies of developmental biology since they are external fertilizers and possess a large number of gametes. Speract, a decapeptide released from the egg jelly, induces chemotaxis in *Lytechinus pictus* sea urchin sperm. This peptide triggers several intertwined biochemical and electrophysiological processes that result in intracellular increases in cyclic nucleotides ([cGMP], [cAMP]), pH (pH<sub>i</sub>) and Ca<sup>2+</sup> [Ca<sup>2+</sup>]<sub>i</sub>, as well as membrane potential changes caused by regulated ionic fluxes of K<sup>+</sup>, Na<sup>+</sup> and Ca<sup>2+</sup>. These events lead to [Ca<sup>2+</sup>]<sub>i</sub> fluctuations that control flagellar beating and thus sperm swimming paths. The identity of the transporters associated with the [Ca<sup>2+</sup>]<sub>i</sub> changes required for chemotaxis is still not fully known. CatSper, a sperm exclusive Ca<sup>2+</sup> channel composed of four alpha subunits and five accessory subunits (beta, gamma, delta, epsilon and zeta), which is expressed exclusively in the flagellum has been detected in sea urchin sperm and there is evidence for its involvement in chemotaxis (Seifert *et al.* *Embo J.* 2015; Espinal-Enriquez *et al.* *Sci Rep.* In press).

This work presents initial findings in an endeavor to electrophysiologically characterize CatSper in sea urchin sperm using the patch-clamp technique. Due to the morphology and size of the sea urchin sperm (3-4 μm diameter head and around 50 μm long of the flagella), we have resorted to the technique previously described by Sánchez *et al.* (*FEBS Lett.* 2001), which swells sperm diluting sea water 10 fold, which increases the probability of obtaining high resistance seals to record ion currents. Applying this experimental strategy we are now obtaining patch-clamp recordings in the cell attached configuration and detecting macroscopic ionic currents.

Support acknowledged from Consejo Nacional de Ciencia y Tecnología CONACyT Fronteras 71, PAPIIT/UNAM IN205516 and NIH RO1 HD038082-13 to A. Darszon.

48-POS Board 48

**Structural and Functional Features of the ATP-dependent Potassium Channels in Mammalian Mitochondria**

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By immunoelectron microscopy, in the rat liver and heart mitochondria, two channel proteins related to the ATP-dependent potassium ion transport system were identified. One of the proteins is immunoreactive to KIR and ROMR antibodies and is localized evenly over the mitochondria. The second protein, which has m. m. 57kD, is localized mainly around the periphery in rat heart mitochondria. The electrophysiological properties of the 57kDa channel protein were studied by its reconstruction into BLM. Low concentrations of ATP activate the channel, whereas high concentrations of ATP closed it. It was found that specific antibodies against this protein inhibit the ATP-dependent potassium transport in mitochondria, without any effect on other mitochondrial functions. MALDI protein analysis revealed that the 57kDa channel protein is 50% homologous to the precursor of calreticulin. Immunoblot analysis showed that the studied protein is practically absent in microsomes, where calreticulin is predominantly localized in microsomes. It was found that uridine diphosphate (UDP) is a metabolic activator of this channel. Using the experimental model of myocardial ischemia, it was observed that the intravenous's injection of the precursor of UDP - uridine increases the concentration of UDP and UTP in the rat heart tissue, and causes pronounced anti-ischemic and anti-arrhythmic effects, manifested in a decrease in the necrosis zone, restoration of the heart rhythm, prevention of ATP and creatine phosphate decreases, as well as in the reduction of hydroperoxide in the myocardium of the experimental animals. The effect of uridine is eliminated by the selective inhibitor of the channel – 5-hydroxydecanoate. Uridine also acts as an antihypoxic factor in other models of oxidative stress. The work was supported by grants from the Government of RF (14.z50.31.0028), and partially from RNF (№ 16-15-00157), and RFBR (№ 16-04-00692a).

**51-POS      Board 51****Linking Deprotonation to Channel Closure for Acid-sensing Ion Channels****Maria Musgaard.**

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Acid-sensing ion channels (ASICs) are proton-gated sodium channels found throughout the central and peripheral nervous systems and e.g. involved in pain signaling. ASICs open upon acidification of the synaptic cleft. At least three states are involved in the functional cycle; the closed (deprotonated), open (protonated), and desensitized (closed, protonated) states. Several crystal structures for the protonated states have been solved, showing a trimeric channel with a large extracellular domain (ECD) and a smaller transmembrane domain (TMD). The structure of the deprotonated closed state is, however, still unknown. Thus, we lack structural understanding of the coupling mechanism between protonation/deprotonation and channel opening/closure. Two ECD proton-sensing regions have been suggested, around 20 Å and 60 Å from the TMD, respectively, and our aim is to gain insight into how these proton sensors couple to channel gating.

We have studied the influence of different protonation states, corresponding to different pH values, on the overall protein dynamics by performing atomistic molecular dynamics simulations. Our initial results, only including the ECD, illustrate that the isolated ECD is structurally stable, and that deprotonation promotes motions in the ECD-TMD linker regions which would cause channel closure in a full-length model with linker distances corresponding to crystal structures of the desensitized state. On the contrary, for the protonated state the linker regions remain separated, corresponding to the open state crystal structures. Changes around the so-called finger-thump site upon deprotonation are observed, as expected for a proton-sensing region, and our data suggest that this might lead to some overall changes in the flexible finger domain.

We are currently investigating whether the full channel responds similarly to deprotonation, and thus whether we can explain the coupling mechanism that links deprotonation to channel closure and predict the structure of the deprotonated state.

54-POS Board 54

**Visualizing Calcium Nanodomains in Living Cells through Ion Channel Optical-Recordings.**

**Jose P. Ocelotl-Oviedo**<sup>1</sup>, Adán O. Guerrero-Cárdenas<sup>2</sup>, Jose D. Martínez-Reyes<sup>3</sup>, Ángel G. Velasco-Félix<sup>2</sup>, Juan García-Rincon<sup>2</sup>, Carlos E. Batián-Eugenio<sup>4</sup>, Luis Vaca-Domínguez<sup>4</sup>, Alberto Darszon<sup>1</sup>.

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Calcium plays a pivotal role in cell physiology as it acts as a second messenger in many signaling processes. Calcium signaling is tightly regulated through the generation of highly localized signals. Single channel activity has been widely studied using patch clamp techniques and a variety of single Ca<sup>2+</sup> channel activities studied. Nevertheless, cells with complex morphologies and Ca<sup>2+</sup> channels with very small single channel conductances (i.e. SOCs) still present a challenge for patch clamp techniques. We present a non-invasive methodology, based on the optical recordings of fluorescent calcium probes using total internal reflexion microscopy which allows studying single Ca<sup>2+</sup> channel activity in living cells. Furthermore, we extended the theory of non-stationary noise analysis of macroscopic currents, developed by (Sigworth. J. Physiol. 307:97. 1980), to provide reliable estimates of the number of ion channels in optical patch clamp recordings. Theoretical (Markov Chain Models of single channel activity) and experimental studies of optical path clamp recordings of Ca<sup>2+</sup> channels (genetically encoded P2X4 fluorescent channels) are presented as case studies.

Acknowledgements: This work was supported by CONACyT and PAPIIT/UNAM to LV, AD and AG.

The results will be discussed.

57-POS Board 57

**Computational Investigation of Hexamer and Tetramer Assemblies of Orai Subunits****Tugba N. Ozturk**<sup>1,2</sup>, Guillaume Lamoureux<sup>1,2,3</sup>.<sup>1</sup>Concordia University, Montreal, QC, Canada, <sup>2</sup>Concordia University, Montreal, QC, Canada,<sup>3</sup>Concordia University, Montreal, QC, Canada.

In many cell types, the depletion of intracellular calcium stores triggers a specific form of calcium influx called store-operated calcium entry, which is crucial for the activation of immune cells and the function of several cell types. In mast cells, store-operated calcium entry is carried by calcium release-activated calcium (CRAC) channels. Orai proteins form the pore subunit of CRAC channels whereas Stromal interaction molecules (STIM) act as sensors for the intracellular calcium stores.

Although it has been suggested that the functional form of CRAC channels is a tetramer assembly of Orai subunits, the recent crystal structure of the Orai protein [Hou et al., *Science* 338, 1308 (2012)] exhibits a hexamer assembly with a threefold, quasi-sixfold axial symmetry around the ion pore of the channel [Thompson and Shuttleworth, *Sci. Rep.* 3, 1961 (2013)]. In addition, the open state of Orai channels is unknown.

In this study, we analyzed the stability and the packing of hexamer and tetramer Orai calcium channels using all-atom molecular dynamics simulations. We modelled the tetramer assembly of Orai subunits based on the hexameric crystal structure. Our results indicate that the tetramer Orai model retains most of the structural features of the hexamer channel, even though the residue-residue contact analysis reveals a packing less tight than for the Orai hexamer. The tetramer channel displays a narrower pore containing fewer water molecules, which affects the solvation of permeant ions. We also computed the free energy profiles of calcium, potassium and sodium ions permeating through the pore of Orai channel models built with the pore-lining helices in conformations suggested to be the active form of the channel [Yamashita et al., *Nat Commun.* 8, 14512 (2017)].

**60-POS      Board 60****Molecular Determinants of Ion Permeation and Selectivity in Voltage-gated Bacterial Sodium Channel Na<sub>v</sub>Ab**Christopher Ing<sup>1,2</sup>, Nilmadhab Chakrabarti<sup>1</sup>, Ning Zheng<sup>3,4</sup>, William A. Catterall<sup>3</sup>, **Régis Pomès**<sup>1,2</sup>.<sup>1</sup>Hospital for Sick Children, Toronto, ON, Canada, <sup>2</sup>University of Toronto, Toronto, ON, Canada, <sup>3</sup>University of Washington, Seattle, WA, USA, <sup>4</sup>Howard Hughes Medical Institute, Seattle, WA, USA.

The determination of high-resolution structures of voltage-gated Na<sup>+</sup> channels has opened the way to elucidating the mechanism of sodium permeation and selectivity. Molecular simulation studies of bacterial sodium channel Na<sub>v</sub>Ab suggest that Na<sup>+</sup> binding and permeation through the selectivity filter are coupled to the conformational isomerization of the Glu177 side chains of the EEEE ring from an out-facing conformation to a lumen-facing conformation, resulting in a high rate of Na<sup>+</sup> diffusion through the selectivity filter. To clarify the role of channel dynamics on ion permeation and selectivity, we examine the mechanism of Na<sup>+</sup> and K<sup>+</sup> permeation in various systems in which either the nature of the EEEE ring or the extent of channel fluctuations have been modified. Extensive molecular dynamics simulations performed in the presence of NaCl, KCl, or a mixture of the two, show how modifying the structure and fluctuations of the selectivity filter alters the conduction mechanism and the competitive binding of Na<sup>+</sup> and K<sup>+</sup>. These findings suggest that conformational fluctuations of the EEEE ring play an important role in the ionic selectivity of voltage-gated Na<sup>+</sup> channels.

**63-POS Board 63****Leak Current in a Mutant Sodium-potassium Pump Found in a Patient with Hypokalaemic Periodic Paralysis**

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Hypokalaemic periodic paralysis (HypoPP) is a rare neuromuscular disease characterized by disabling episodes of flaccid skeletal muscle paralysis, lasting hours to days, with accompanying low serum K<sup>+</sup> levels. The disease can progress to a permanent proximal myopathy. The general consensus is that arginine-neutralizing mutations in the voltage sensor of NaV1.4 or CaV1.1 sarcolemmal ion channels lead to gating pore leak currents through their voltage sensing domains. These currents depolarize the muscle and reduce excitability. We identified a young boy who presented with recurrent episodes of quadriparesis and low serum K<sup>+</sup> consistent with a diagnosis of HypoPP who did not carry mutations in known HypoPP genes but instead a novel mutation in the ATP1A2 gene, encoding the alpha 2 subunit of the Na<sup>+</sup>/K<sup>+</sup> ATPase. Creatine kinase was elevated and a muscle biopsy myopathic, confirming a muscle pathology. The patient also showed CNS symptoms (seizures and mild learning difficulties), consistent with alpha 2 expression in astrocytes. The missense mutation affects a serine residue (S779) in the K<sup>+</sup> binding site of the pump, with predicted deleterious functional consequences. Electrophysiological characterization of pump activity was performed using two electrode voltage clamp in *Xenopus laevis* oocytes. The effects of the mutation were two-fold. 1. The mutant ATPase carries an abnormal ouabain-sensitive leak current analogous to the omega currents known to cause HypoPP. 2. The mutant ATPase presents reduced K<sup>+</sup> affinity, resulting in a lower turnover rate at physiological K<sup>+</sup> concentrations. The leak current is carried by protons and potentially Na<sup>+</sup> ions. This is the first report of a leak current through a membrane protein other than voltage-gated ion channels as a patho-mechanism of HypoPP, and may warrant re-evaluation of the HypoPP classification and genetic diagnosis practices.

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**Gating the CLC-2 Chloride Channel by Permeant Anions****Jorge Sanchez Osuna**<sup>1</sup>, Jorge Arreola Gómez<sup>1</sup>, Arlette Méndez Maldonado<sup>2</sup>,<sup>1</sup>Autonomous University of San Luis Potosí, San Luis Potosí, San Luis Potosí, Mexico,<sup>2</sup>Autonomous University of San Luis Potosí, San Luis Potosí, San Luis Potosí, Mexico.

The activation of the homodimeric CLC-2 Cl<sup>-</sup> channel is voltage dependent; however, the channel lacks classical voltage-sensing domains. The closed to open transition is initiated by dislodging the negatively charged side chain of an acid glutamic residue (E213), which constitutes the gate located inside the pore. We hypothesize that a hyperpolarization introduces intracellular anions into the pore and forces the gate to open to allow anion permeation. In the present work, we analyze the gating mechanism of CLC-2 using experimental and computational electrophysiology. We determine the effects of different concentrations of intracellular acetate (0, 20, 60, 80 mM), a low permeant anion (P<sub>acetate</sub>/P<sub>Cl</sub> = 0.04), mixed with 60 mM Cl<sup>-</sup>. In addition, intracellular Cl<sup>-</sup> concentrations (0, 20, 40, 60 mM) mixed with 80 mM acetate. Cells were bathed in a solution containing 140 mM Cl<sup>-</sup> and voltage dependent activation was evaluated by measuring the tail current at +60 mV after channel activation in the range of +60 to -200 mV. We found that increasing the acetate concentration from 0 to 80 mM shifted channel activation from -140.2 mV to -87.4 mV. In contrast, increasing the intracellular Cl<sup>-</sup> from 0 to 60 mM shifted the channel activation from -110.4 mV to -69.5 mV. We concluded that activation was mediated by multi-ion occupancy and that the pore occupation by anion is a necessary step to open the gate. To infer the molecular events leading to channel opening by the anion-gate interaction, we used molecular dynamics simulation (computation electrophysiology). We embedded CLC-2 into a dipalmitoyl-phosphatidylcholine membrane and exposed to symmetrical 140 mM NaCl. A voltage difference of -140 mV drive two Cl<sup>-</sup> ions from the intracellular side into the pore. The leading Cl<sup>-</sup> interacts electrically with E213 side chain to unblock the conduction pathway.

**69-POS**      **Board 69****Molecular Insights into Kv1.2 Channel Modulation by General Anesthetic Sevoflurane****Leticia Stock**, Juliana Hosoume, Werner Treptow.

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Anesthetics have been routinely used in medical procedures for almost two centuries. Every year millions of people undergo surgery with anesthesia, attesting to its irrevocable importance to modern medicine. Despite its role in health and relatively safe administration, the molecular mechanism leading to endpoint of anesthesia remains unknown. Early propositions argued anesthetics would act by altering cellular membranes' physicochemical properties. Nonetheless, studies favoring allosteric modulation of multiple proteins targets are now challenging the indirect membrane-mediated hypothesis. One such target, evidenced by electrophysiology and *in vivo* experiments, is voltage-gated channel Kv1.2. Investigations on sevoflurane, a major general anesthetic, suggest it potentiates Kv1.2 by binding to multiple independent sites, causing a left-shift to the conductance-per-voltage (GV) curve, while also increasing its maximum conductance.

We wish to identify Kv1.2 sevoflurane binding sites and quantify to what extent can macroscopically measured channel potentiation be recovered from direct modulation. For that, we've developed a theoretical framework to investigate small ligand concentration-dependent interaction to multiple saturable binding sites which allow for thorough calculation of the functional impact of such binding to equilibrium between well-known conformational states, i.e. open and closed Kv1.2 structures. Local anesthetic distribution and binding affinities are evaluated by a combination of docking and free-energy-perturbation calculations.

We find that sevoflurane binds Kv1.2 in a conformation-dependent manner. Also, the calculated open-conformation stabilization effected by the ligand agrees with experimental measurements. Our results successfully recover GV leftward shift from microscopic data alone. Key binding sites identified by the docking-FEP strategy are found to be in close proximity to residues identified as relevant by recent photolabeling and mutagenesis experiments. Altogether, results support the direct modulation hypothesis and contributes to understanding sevoflurane effects from a molecular standpoint. The theory is also general and could be applied to various ligand-receptor systems.

72-POS      Board 72

**Probing the Mechanosensitive Domains of Piezo Ion Channels with Magnetic Force**

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Piezo ion channels are excitatory mechanically activated channels that mediate our sense of touch as well as a number of other roles in pressure sensing organs throughout the body. However, the precise mechanisms and structures mediating the processes of mechanical activation and subsequent inactivation are still unclear. To address these questions, we use magnetic nanoparticles as localized force transducers that allow for remote mechanical stimulation of different domains within Piezo1. In combination with pressure-clamp electrophysiology, we use magnetic pulling forces to identify distinct domains that affect channel activation and inactivation. Revealing these domains supports our future mechanistic studies for understanding Piezo function.