CANAUX IONIQUES

# 34th Ion Channel Meeting

## 14th-17th September 2025
## Domaine du Lazaret, Sète, France

**Plenary lecture**
**John Wood (London, United Kingdom)**

**Innovations and trends in channelopathies**
Jamie Vandenberg (Darlinghurst, Australia)
Rupamanjari Majumder (Nantes, France)
Pau Gorostiza Langa (Barcelone, Spain)

**Ion signaling and cell bioenergetics in cancer**
Ildiko Szabo (Padova, Italy)
Geert Bultynck (Leuven, Belgium)
Ivan Bogeski (Göttingen, Germany)

**Secondary ion channel dysfunction in pathophysiological processes**
Joost Hoenderop (Nijmegen, The Netherlands)
Karin Sipido (Leuven, Belgium)
Heinz Beck (Bonn, Germany)

**Ion channels in plants and fungi**
Schroeder Julian (La Jolla CA, USA)
Alexis De Angeli (Montpellier, France)
Anthony Lewis (Portsmouth, United Kingdom)

**Genetically encoded pharmacology in ligand-gated ion channels**
Shujia Zhu (Shenzen, China)
Shai Berlin (Haifa, Israel)
Marie Prévost (Paris, France)

# CONTENTS

# ORGANIZING AND SCIENTIFIC COMMITTEE

**Cécile HILAIRE (chair)**
INM INSERM U1298, Université de Montpellier
cecile.hilaire@umontpellier.fr

**Claire LEGENDRE (chair)**
Laboratoire MitoVasc, UMR CNRS 6015 - INSERM U1083, Université d'Angers
claire.legendre@univ-angers.fr

**Alban GIRAULT**
Laboratoire de Physiologie Cellulaire et Moléculaire, Université de Picardie, UR UPJV 4667
alban.girault@u-picardie.fr

**Barbara RIBEIRO DE OLIVEIRA**
Nantes Université, CNRS, INSERM, L'institut du thorax
barbara.ribeiro@univ-nantes.fr

**Vincent SEUTIN**
Neurophysiology Lab, GIGA Neurosciences, Liège University, Belgium
v.seutin@uliege.be

**Laetitia MONY**
IBENS, ENS-PSL Université, CNRS UMR 8197, INSERM U1024
laetitia.mony@bio.ens.psl.eu

**Jean-Sébastien ROUGIER**
Privat Dozent, IBMM, University of Berne, Suisse
jean-sebastien.rougier@unibe.ch

**Jérôme THIREAU**
Laboratoire PhyMedExp, Montpellier
jerome.thireau@inserm.fr

**Valerio FARFARIELLO**
Laboratoire PhyCell, INSERM UMR1003, Université de Lille
valerio.farfariello@univ-lille.fr

**Adèle FAUCHERRE**
IGF, CNRS UMR5203, INSERM U1191, Université de Montpellier
adele.faucherre@igf.cnrs.fr

**Loïc LEMONNIER**
Laboratoire PhyCell, INSERM UMR1003, Université de Lille
loic.lemonnier@inserm.fr

# ION CHANNELS ASSOCIATION

**Arnaud MONTEIL (President)**
Institut de Génomique Fonctionnelle, CNRS UMR 5203 - INSERM U1191
Université de Montpellier,
arnaud.monteil@igf.cnrs.fr

**Caroline STRUBE (vice President)**
Centre de Recherche en Neurobiologie et Neurophysiologie de Marseille, CNRS UMR 7286,
Université Aix Marseille,
Caroline.strube@univ-amu.fr

**Claire LEGENDRE (Treasurer)**
Laboratoire MitoVasc, UMR CNRS 6015 - INSERM U1083, Université d'Angers
claire.legendre@univ-angers.fr

**Cécile HILAIRE (Deputy Treasurer)**
INM INSERM U1298, Université de Montpellier
cecile.hilaire@umontpellier.fr

**Alban GIRAULT (Secretary)**
Laboratoire de Physiologie Cellulaire et Moléculaire, Université de Picardie, UR UPJV 4667
alban.girault@u-picardie.fr

## SPONSORS – EXHIBITORS- ADVERTISERS

**We are very grateful to the following sponsors for their financial and material support.**

# FOREWORD

***Dear Colleagues,***

*We are delighted to welcome you once again to Sète for the 34th edition of the Ion Channels Meeting. This year's program reflects the vitality and diversity of ion channel research, with contributions ranging from fundamental mechanisms to translational perspectives in physiology and disease. Topics will span multiple areas of biology (cardiac physiology, renal magnesium transport, immunity, and plant and fungal physiology) as well as pathophysiology (pain, arrhythmias, atrial fibrillation and sudden cardiac death, neurodegenerative diseases, encephalitis, epilepsy, cancer, …). The approaches presented are equally varied and at the cutting edge of technology, including functional genomics, computational modeling, AI and machine learning, structural biology, nanobodies, toxin engineering, cryo-EM, and of course electrophysiology.*

*Ion channels occupy a central role in global research, and the French Ion Channel Association is proud to celebrate 34 years of annual meetings. We are grateful for your participation, with this year's edition bringing together 102 registrations and 60 abstract submissions. Invited speakers and participants are joining us from across the world (USA, China, Australia, Israel, United Kingdom, Belgium, Germany, Switzerland, Spain, Italy, Denmark, and the Netherlands), a testimony to the international visibility and vitality of our field.*

*This meeting would not be possible without the support of our sponsors and the dedication and hard work of the organizing committee: Valerio Farfariello, Adèle Faucherre, Alban Girault, Cécile Hilaire, Claire Legendre, Loïc Lemonnier, Laetitia Mony, Barbara Ribeiro Oliveira-Mendes, Jean-Sébastien Rougier, Vincent Seutin, and Jérôme Thireau — not to forget Arnaud Monteil and Caroline Strube, the true guardians of the soul of this congress.*

*We hope that our scientific exchanges will be fruitful and that the Mediterranean setting of the Lazaret will provide the perfect environment to strengthen and promote new collaborations within our network.*

***Enjoy the meeting!***

*Cécile and Claire*

*Presidents of the 34th Ion Channels Meeting*

# PROGRAM

## Sunday, September 14th 2025

**16:00 – 19:00** Welcome of the meeting attendees

**19:00 *Welcome drink and Dinner***

**21:00 Plenary lecture**

**John Wood (University College London, London, UK)**
"Sodium channels and pain"

## Monday, September 15th 2025

**08:30 Opening session**

**08:45 Symposium 1: "Innovations and trends in channelopathies"**
*Organized by Barbara Ribeiro (L'institut du thorax, Nantes, France)*

**Jamie Vandenberg (Victor Chang Cardiac Research Institute, Darlinghurst, Australia)**
"Can ion channel functional genomics assays help predict risk of sudden cardiac death"
**Rupamanjari Majumder (L'institut du thorax, Nantes, France)**
"Computational cardiac optogenetics to investigate mechanisms of cardiac arrhythmias"
**Pau Gorostiza Langa (Institute for Bioengineering of Catalonia, Barcelone, Spain)**
"Controlling the activity of neuronal ion channels and receptors with light: from single synapses to brain waves"

*Selected speaker:*
**Julia Kacher (LPCT, Université de Lorraine)**
"Ai-driven pipeline for predicting the dynamic gating pathways of voltage-gated ion channels"

**10:30 Sponsor presentation**

**10:45 *Coffee break***

**11:15 Oral communication session 1: "Ion channels at heart"**
*Organized by Adèle Faucherre (IGF, Montpellier, France)*

**Anthony Coté (University Medical Center Freiburg, Germany)**
"Organotypic Cardiac Slices from Human Hearts as a Platform for Antiarrhythmic Drug Testing"
**Christopher Beaudoin (University of Oxford, UK)**
"Modelling supramolecular clusters of voltage-gated sodium channels in the cardiomyocyte perinexal space"

**Aline Rio (L'institut du thorax, Nantes, France)**
"Exploration of the HERG channel interactome for a better understanding of inherited cardiac arrhythmias"
**Marvin Gaillardon (Institut de Génomique Fonctionnelle, Montpellier, France)**
"Genetic ablation of L-type CaV1.3 channels in cardiac conduction system induces bradycardia and increased susceptibility to atrial fibrillation"

**12:15 -12:45 1-minute oral presentation for posters (even numbers)**

***12:45 Lunch***

**14:00 Poster session 1**

***15:30 Coffee break***

**15:45 Sponsor presentation**

**16:00 Symposium 2: "Secondary ion channel dysfunction in pathophysiological processes"**
*Organized by Vincent Seutin (Neurophysiology Lab, GIGA Neurosciences, Liège University, Belgium)*

**Joost Hoenderop (Radboud University, Nijmegen, The Netherlands)**
"Novel aspects of renal magnesium transport in health and disease"
**Karin Sipido (Experimental Cardiology, KU Leuven, Leuven, Belgium)**
"Heterogeneity of cardiac ion channels in disease – new insights for arrhythmias"
**Heinz Beck (Life and Brain Center, University of Bonn, Bonn, Germany)**
"Dendritic mechanisms of cognitive comorbidities in epilepsy"

*Selected speaker:*
**Jean-François Desaphy (University of Bari Aldo Moro, Italy)**
"Do CLC-2 chloride channels play a role in human pancreatic ductal adenocarcinoma?"

**17:45 - 18:45 Oral communication session 2: "Ion channel modulation: from molecule identification to physiological effects"**
*Organized by Alban Girault (Laboratoire LPCM, UR UPJV 4667, Amiens, France)*

**Serena Hachem (L'institut du thorax, Nantes, France)**
"Screening and optimization of cell-penetrating peptides for delivery in human cardiomyocytes"
**Nurcan Inci (Neurophysiology Lab, GIGA Neurosciences, Liège University, Belgium)**
"Comprehensive in vitro evaluation of five Nav blockers in two mutants responsible for Paramyotonia Congenita"
**Frans Lisse (Ghent University, Belgium):**
''Cholesterol as a modulator of the IKs channel complex''
**Julien Louradour (University of Bern, Switzerland)**
"Beneficial APD-shortening effects, but deleterious negative inotropism of IKs-activator Docosahexaenoyl glycine (DHA-gly) in long QT syndrome type 2"

18:45 Sponsor presentation

*19:30 Apéritif dinatoire Sétois*

## Tuesday, September 16th 2025

**08:30 Symposium 3: "Ion signaling and cell bioenergetics in cancer"**
*Organized by Valerio Farfariello (Laboratoire PhyCell - INSERM UMR1003, Lille, France)*

**Ildiko Szabo (Department of Biology, University of Padova, Padova, Italy)**
"Multiple roles of mitochondrial ion channels in poor prognosis cancers"
**Geert Bultynck (Department Cellular & Molecular Medicine, KU Leuven, Belgium)**
"BAPTA, an intracellular calcium chelator, and its impact on metabolism & cell death in B-cell cancers: sadly, not at all a story about calcium"
**Ivan Bogeski (University Medical Center Göttingen, Göttingen, Germany)**
"Calcium and redox signals at cellular interfaces: Implications for immunity and cancer"

*Selected speaker:*
**Marina Villaro (Instituto de Neurociencias, UMH-CSIC, Sant Joan d'Alacant, Spain)**
"Cold-activated spinal circuitry and TRP channel contribution in chemotherapy-induced peripheral neuropathy (CIPN)"

*10:15 Coffee break*

**10:30 - 11:00 1-minute oral presentation for posters (odd numbers)**

**11:00 - 12:30 Poster Session 2**

**12:30 Lunch**

*13:30 Appointment for Social event*

**17:15 Symposium 4: "Ion channels in plants and fungi"**
*Organized by Jean-Sébastien Rougier (Institute of Biochemistry and Molecular Medicine, Berne, Suisse)*

**Julian Schroeder (Division of Biological Sciences, La Jolla CA, USA)**
"Identification of the Stomatal CO 2 sensor that Controls Plant Water Loss and Water Use Efficiency"
**Alexis De Angeli (IPSiM, Montpellier, France)**
"Structure and function of plant ion channels"
**Anthony Lewis (Ion Channel Research Group, Portsmouth, United Kingdom)**
"Gating and regulation of fungal potassium channels"

*Selected speaker:*
**Elsa Demes-Causse (Institut des Sciences des Plantes de Montpellier, France)**
"Vacuolar malate and fumarate transport through ATALMTs"

**19:00 Annual meeting of the Association**

***20:00 Dinner***

***21:00 Relaxing Presentation***

**Artem Kondratskyi (Villeneuve-d'Ascq, France)**
"Amazing stories of ion channels in action"

## Wednesday, September 17th 2025

**09:30 Symposium 5: "Genetically encoded pharmacology in ligand-gated ion channels"**
*Organized by Laetitia Mony (Institute of Biology of Ecole Normale Supérieure, Paris, France)*

**Shujia Zhu (Southern University of Science and Technology, Shenzhen, China)**

Native NMDA receptors in the brain: from atomic structure to synaptic physiology
**Shai Berlin (Technion, Haifa, Israel)**
Genetically-encoded toxins acting on Kv channels and NMDA receptors
**Marie Prévost (Institut Pasteur, Paris, France)**
Mechanisms of nanobody modulation of a pentameric channel-receptor

*Selected speaker:*
**Jennifer D. Noonan (Humboldt Universität zu Berlin, Berlin, Germany)**
"Making an AMPA receptor slow"

***11:15 Coffee break***

**11:40 Prizes and Meeting closure**

***12:00 Lunch***

# SYMPOSIA AND ORAL COMMUNICATIONS ABSTRACTS

## Sunday, September 14$^{th}$ 2025

### 21:00 - Keynote lecture

**SODIUM CHANNELS AND PAIN**

**John Wood**
*Molecular Nociception Group, University College London (UCL), London, UK*

Voltage-gated sodium channels underpin electrical signalling in sensory neurons. Their activity is an essential element in the vast majority of pain conditions, making them significant drug targets. Sensory neuron sodium channels play roles not only in afferent signalling but also in a range of efferent regulatory mechanisms, interacting with the immune system. The fact that sodium channel blockers such as lidocaine are effective analgesics focussed attention on the three sensory neuron isoforms, NaV1.7–NaV1.9, foat high levels in the peripheral nervous system. Importantly, Nav1.7, Nav1.8 and Nav1.9 null mutant mice are viable but show deficits in pain behaviour, making them attractive drug targets. In fact, only NaV1.8 is selectively expressed in sensory neurons, while NaV1.7 and NaV1.9 are found in a variety of CNS and PNS neurons as well. Nav1.7 sensory neuron null mice and humans are pain-free, making this channel a top Pharma target for the past two decades. However, embryonic nulls mask a potentially lethal phenotype by compensatory expression of other channels. Nav1.7 antagonists act on both sensory and sympathetic neurons to cause catastrophic side effects, so Nav1.7 antagonists have no therapeutic window and are being abandoned. In contrast Nav1.8 antagonists mimic the phenotype of Nav1.8 nulls. In a tour-de-force, the recent development of an orally active Nav1.8 antagonist named Journavx (Suzetrigine) provides a remarkable advance in pain treatment and has FDA approval. Unlike other analgesics, Journavx targets electrical activity in sensory neuron axons, acting rather like lidocaine - but specifically targeting damage-sensing neurons (nociceptors) rather than all aspects of somatosensation. This unique site of action suggests that alone or in combination with other analgesics, Journavx is likely to provide opportunities for pain relief for the vast number of people (about a third of the population) impacted by chronic pain. Given the present opioid crisis in the United States, this is a very welcome development. Today we will discuss in detail aspects of this clinically significant scientific advance.

# Monday, September 15$^{th}$ 2025

## 08:45 - **Symposium 1: “Innovations and trends in channelopathies”**

Organized by Barbara Ribeiro (L’institut du thorax, Nantes, France)

**CAN ION CHANNEL FUNCTIONAL GENOMICS ASSAYS HELP PREDICT RISK OF SUDDEN CARDIAC DEATH?**

**Jamie Vandenberg**

*Victor Chang Cardiac Research Institute, Darlinghurst, Australia*

A range of inherited cardiac arrhythmia syndromes are caused by variants in cardiac ion channel genes. These syndromes, including LQTS and Brugada syndrome, are characterized by highly variable clinical presentation, ranging from severe arrhythmias and death in utero to no overt symptoms well into adult life. The aim of our work is to investigate to what extent disease severity is influenced by how different genetic variants influence channel function. We have developed high throughput patch clamp assays to assess the functional impact of a wide range of variants in ion channel genes. First, we determined the sensitivity and specificity of our assays for detecting known pathogenic and known benign variants and established thresholds for determining the likelihood that any given variant should be classified as (likely) benign or (likely) pathogenic. We then used cox-proportional hazard modelling to investigate whether the severity of loss of function in KCNH2 or SCN5A variants correlated with clinical outcomes. Our results show that For KCNH2-related LQTS, the extent of loss of function as determined by a high throughput patch clamp assay, provides independent predictive value for risk of severe cardiac events.  In SCN5A-related Brugada syndrome, the extent of loss of function of SCN5A activity, was the best single predictor of penetrance.

**COMPUTATIONAL CARDIAC OPTOGENETICS TO INVESTIGATE MECHANISMS OF CARDIAC ARRHYTHMIAS**

**Rupamanjari Majumder**

*L’Institut du thorax, Nantes, France*

Cardiac optogenetics is an emerging technology that enables the control of cardiac myocytes—normally insensitive to light—by genetically introducing light-sensitive ion channels. This allows precise modulation of the cells' electrical activity using light of specific wavelengths. The approach holds remarkable promise in therapeutic applications, primarily because it harnesses endogenous ionic currents and operates at very low energy levels—potentially offering a non-invasive and painless method of intervention. While there is ongoing debate about its clinical applicability, due to both technical and translational challenges, the technology undeniably opens exciting new avenues from a research perspective. Most notably, cardiac optogenetics provides unprecedented spatiotemporal control, making it possible to investigate nonlinear dynamics in cardiac tissue with a level of precision that traditional electrophysiological methods cannot achieve. In this talk, I will present an overview of the groundbreaking research enabled by cardiac optogenetics, highlight the current state of the art, and explore the boundaries of what this technology may ultimately achieve.

## CONTROLLING THE ACTIVITY OF NEURONAL ION CHANNELS AND RECEPTORS WITH LIGHT: FROM SINGLE SYNAPSES TO BRAIN WAVES

**Pau Gorostiza**
*Catalan Institution for Research and Advanced Studies (ICREA), Institute for Bioengineering of Catalonia, the Barcelona Institute of Science and Technology (IBEC), Network Biomedical Research Center on Bioengineering, Biomaterials & Nanomedicine (CIBERbbn)*

The large number of photoswitchable biomolecules discovered and developed in recent years covers a great variety of cellular functions like catalysis of metabolic processes, cytoskeletal polymerization and motors, nucleic acids dynamics, intracellular signaling and perhaps most dazzlingly membrane excitability, which has been at the focus of photopharmacology and optogenetics to study neurobiology. The dream of precisely and remotely photocontrolling every aspect of neuronal activity in intact tissue appears within reach and offers the promise of understanding the underlying molecular mechanisms [1]. Recent and ongoing projects at IBEC focused on photopharmacology will be outlined, including the development and applications of photoswitchable ligands of ion channels and receptors to control neuronal activation and inhibition with one-, two-, and three-photon excitation [2]. These molecular tools allow spatiotemporal control of endogenous proteins in vivo [2-7] at multiple scales from emerging cortical waves in the brain [8, 9] to individual synapses [10, 11]. They also enable multiple applications, from sensory restoration [4] to noninvasive inhibition of pain [6].

**References**

[1] Sahu S, Amrutha AS, Tamaoki N. Med Res Rev. 2025. doi: 10.1002/med.22106.
[2] Sortino R, Cunquero M, Castro-Olvera G, Gelabert R, Moreno M, Riefolo F, Matera C, Fernàndez-Castillo N, Agnetta L, Decker M, Lluch JM, Hernando J, Loza-Alvarez P, Gorostiza P. Angew Chem Int Ed Engl. 2023; 62:e202311181. doi: 10.1002/anie.202311181.
[3] Castagna R, Maleeva G, Pirovano D, Matera C, Gorostiza P. J Am Chem Soc. 2022; 144:15595-15602. doi: 10.1021/jacs.2c04920.
[4] Garrido-Charles A, Huet A, Matera C, Thirumalai A, Hernando J, Llebaria A, Moser T, Gorostiza P. J Am Chem Soc. 2022;144:9229-9239. doi: 10.1021/jacs.1c12314.
[5] Matera C, Calvé P, Casadó-Anguera V, Sortino R, Gomila AMJ, Moreno E, Gener T, Delgado-Sallent C, Nebot P, Costazza D, Conde-Berriozabal S, Masana M, Hernando J, Casadó V, Puig MV, Gorostiza P. Int J Mol Sci. 2022; 23:10114. doi: 10.3390/ijms231710114.
[6] Camerin L, Maleeva G, Gomila AMJ, Suárez-Pereira I, Matera C, Prischich D, Opar E, Riefolo F, Berrocoso E, Gorostiza P. Angew Chem Int Ed Engl. 2024 Sep 16;63(38):e202403636. doi: 10.1002/anie.202403636.
[7] Maleeva G, Nin-Hill A, Wirth U, Rustler K, Ranucci M, Opar E, Rovira C, Bregestovski P, Zeilhofer HU, König B, Alfonso-Prieto M, Gorostiza P. J Am Chem Soc. 2024; 146:28822-28831. doi: 10.1021/jacs.4c08446.

[8] Barbero-Castillo A, Riefolo F, Matera C, Caldas-Martínez S, Mateos-Aparicio P, Weinert JF, Garrido-Charles A, Claro E, Sanchez-Vives MV, Gorostiza P. Adv Sci. 2021;8:e2005027. doi: 10.1002/advs.202005027.
[9] Sanchez-Sanchez JM, Riefolo F, Barbero-Castillo A, Sortino R, Agnetta L, Manasanch A, Matera C, Bosch M, Forcella M, Decker M, Gorostiza P, Sanchez-Vives MV. PNAS Nexus. 2025; 4:pgaf009. doi: 10.1093/pnasnexus/pgaf009.
[10] Santini R, Maleeva, G et al., submitted (2025).
[11] Garrido-Charles A, Bosch, M et al., submitted (2025).

## AI-DRIVEN PIPELINE FOR PREDICTING THE DYNAMIC GATING PATHWAYS OF VOLTAGE-GATED ION CHANNELS

**Julia Kacher**, Mounir Tarek
*LPCT, Université de Lorraine*

While high-resolution structures from techniques like cryo-electron microscopy have become increasingly available, understanding the dynamic function of voltage-gated ion channels (VGICs) remains a fundamental challenge. The transient intermediate conformations that connect resting and activated states are critical for defining a channel's unique gating properties and its role in disease, but their fleeting nature makes them difficult to resolve experimentally. While molecular dynamics (MD) simulations can model these transitions, they are often limited by immense computational costs and the difficulty of overcoming energy barriers within feasible timescales. To overcome these limitations, we have developed a novel deep learning pipeline that integrates AI with physics-based insights to efficiently predict the intermediate states of VGICs. Our approach employs a 1D convolutional autoencoder that learns a low-dimensional, interpretable representation of a channel's conformational landscape from structural data of two distinct operational states, such as those from short MD simulations. By navigating this learned representation, our model generates novel structures, effectively mapping the transition pathway between the initial states. A key innovation of our pipeline is the loss function tailored to voltage-gated ion channels. This function incorporates not only traditional geometric error but also physics-based energy constraints and novel terms related to the channel's gating charge. Recognizing gating charge as a superior collective variable for this protein superfamily ensures our predictions are not just structurally plausible but also biologically meaningful. We have successfully validated this pipeline on the Kv1.2 channel voltage sensor domain and extended its application to the whole tetrameric Kv7.1 channel, a critical player in cardiac function. Our research provides a powerful computational tool to generate testable hypotheses about VGIC function. The predicted dynamic pathways offer crucial insights into how patient-derived mutations, implicated in channelopathies like epilepsy and cardiac arrhythmias, can alter gating mechanisms.

## 11:10 - **Oral communication session 1: “Ion channels at heart”**

Organized by Adèle Faucherre (Institut de Génomique Fonctionnelle, Montpellier, France)

### ORGANOTYPIC CARDIAC SLICES FROM HUMAN HEARTS AS A PLATFORM FOR ANTIARRHYTHMIC DRUG TESTING

**Anthony Coté**, Jayce Phillips, Peter Kohl, Ursula Ravens, Remi Peyronnet
*Institute of Experimental Cardiovascular Medicine, University Medical Center Freiburg, Germany*

Introduction: Organotypic cardiac slices provide a promising approach for short- and long-term drug screening. Our aim is to validate this model by testing the electrophysiological effects of standard antiarrhythmic drugs: flecainide (class 1c antiarrhythmic) and dofetilide (class 3 antiarrhythmic) in slices of human hearts. Methods: Left ventricular tissue excised from patients was sliced into 400 µm thick sections, mounted in biomimetic chambers, and paced electrically at a frequency of 1 Hz. A preload of 1 mN was applied. Action potentials (AP) were recorded at 37°C using intracellular microelectrodes pulled from borosilicate glass capillaries (tip resistance 15-20 MΩ when filled with 3 M KCl solution). The bath solution contained (in mM): NaCl 140, KCl 5.4, HEPES 10, CaCl2 1.8, MgCl2 1, glucose 10. Observations before, during and after drug interventions are all paired. All values are reported as mean ± standard deviation. Results: AP from slices had resting membrane potentials (RMP) of -83.7 ± 5.5 mV, AP amplitudes (APA) of 110.4 ± 7.1 mV, maximal depolarization speeds (dV/dtmax) of 104.3 ± 45.7 mV/ms, AP durations at 30% of repolarization (APD30) of 214.6 ± 24.0 ms, APD50 of 327.2 ± 58.1 ms and APD90 of 460.1 ± 72.4 ms (N=8 hearts, n=18 slices). The triangulation index was measured at 1.17±0.26. Thus, AP exhibited consistent shapes during phase 3 though the APD50 and APD90 values varied considerably among patients. Flecainide caused a reversible decrease in dV/dtmax of 25.8 ± 13.2% at 3 µM (N=3, n=6) while APD90 was not significantly altered. Dofetilide reversibly increased APD90 by 22.2 ± 3.2% at 10 nM, and by 50.1 ± 3.9% at 30 nM (N=1, n=2). Conclusion: The organotypic cardiac slice model produced consistent results with regard to RMP, APA values, and AP shape showed clear triangularisation. Although other AP parameters, such as dV/dtmax and individual APD varied among patients, known antiarrhythmic drugs tested produced the expected effects. The results obtained in this study suggest that the slice model is suitable for testing of drug effects on key parameters of cardiac electrophysiology, making this model promising for assessing novel antiarrhythmic drugs.

### GENETIC ABLATION OF L-TYPE CAV1.3 CHANNELS IN CARDIAC CONDUCTION SYSTEM INDUCES BRADYCARDIA AND INCREASED SUSCEPTIBILITY TO ATRIAL FIBRILLATION

**Marvin Gaillardon**, Mélanie Faure, Audrey Dubourg, Isabelle Bidaud, Kai Schönig, Dunan Bartsch, Matteo Mangoni
*Institut de Génomique Fonctionnelle, Université de Montpellier, France*

The spontaneous activity of pacemaker cells in sinoatrial node (SAN) controls the heart rhythm under physiological conditions. Several studies by our group show the relevance of the L-type Ca2+ channel Cav1.3 on the generation of pacemaker activity and in electrical conduction system within the heart. Atrial fibrillation (AF) is the most frequent cardiac arrythmia, often associated with an electrical remodeling. So far, the underlying electrophysiological mechanism is not fully understood. Here we aim to evaluate the impact of global genetic Cav1.3 deletion or its selective deletion in the conduction system, on the AF susceptibility. We generated mice carrying conditional floxed Cav1.3 allele (Cav1.3 Flex eGFP / Flex eGFP) and crossed with HCN4 CreERT2 line allowing deletion of Cav1.3 in cardiac HCN4 expressing cells (HCN4+) upon tamoxifen injection (Cav1.3 eGFP/eGFP mice) within conduction system. The second mice model is a Cav1.3 KO allowing a complete deletion of Cav1.3 in all cardiac tissue. We investigated the effect of Cav1.3 deletion on heart automaticity by telemetric ECGs and Langendorff-perfused hearts. Then, susceptibility to AF was study by using a Langendorff-perfused heart coupled to optical mapping. S1-S9 train pulses stimulation protocols were used to trigger AF. Cav1.3 eGFP/eGFP mice displayed in vivo significant bradycardia (464± 35 BPM in untreated group vs 377±31BPM in tamoxifen group) as well as ex vivo (411±50 BPM vs. 278±78 BPM), associated with an increase of AF susceptibility (22% in untreated group vs 56% in tamoxifen group) compared to untreated group. Similarly, the global deletion of Cav1.3 in the heart lead to slower automaticity (348±74 BPM in WT mice vs. 224±55 BPM in Cav1.3 KO) and increase arrythmias such as atrio-ventricular (AV) blocks and AF susceptibility. In sum, our data show that deletion of Cav1.3 leads to impaired electrical conduction and heart rate generation. Loss of Cav1.3 causes major rhythms disorders such as bradycardia increased heart rate variability, AV blocks, and seems to increase significantly AF susceptibility. This study underscores the major role of Cav1.3 in cardiac conduction system. Thus, Cav1.3 channel could be a relevant target to improve AF treatment.

## MODELLING SUPRAMOLECULAR CLUSTERS OF VOLTAGE-GATED SODIUM CHANNELS IN THE CARDIOMYOCYTE PERINEXAL SPACE

**Christopher Beaudoin**, Christopher Huang, Antony Jackson
*Department of Biochemistry, University of Cambridge, UK*

The members of the voltage-gated sodium channel family (Nav1.1-1.9) are known to carry action potentials along nerve and muscle fibers. A Nav channel comprises a single chain of four internally homologous domains that enclose a central pore, which determines ion selectivity and passage dynamics. Additionally, each channel domain contains a voltage sensing domain (VSD) that allosterically regulates the tertiary structure – resulting in the opening and closing of the selectivity pore – in response to changes in membrane voltage. Four accessory β-subunits (β1-4) have been discovered to interact with Nav channels to modulate voltage gating, kinetics, trafficking, and localization. The β-subunit structures are characterized by an N-terminal extracellular Ig-like domain, a transmembrane domain, and a C-terminal intracellular tail – all of which been shown to interact with Nav channels. Previous findings by the Jackson and other groups have revealed that the Ig-like domains of the β-subunits can oligomerize while the transmembrane domains may, nevertheless, bind to the Nav channels. Furthermore, our recently published data suggest that N-linked glycans on Nav1.5, which is primarily expressed in cardiomyocytes, may prevent the β1 and β3 Ig-like domains from interacting with the channel and induce β-subunit oligomerization while the transmembrane domains may still interact. Therefore, the binding of β-subunit oligomers to Nav1.5 may form networks of channels that permit specialized and localized action potential generation in cardiomyocyte membranes – particularly in the perinexal space. Using protein structure and plasma membrane modelling and molecular dynamics simulations, the interactions between Nav channels/β-subunit clusters on the same and opposing membranes were investigated to shed light on the structural and electrophysiological effects of such multimerization. These modelling efforts provide novel insights into the perinexal space and its effect on action potential conduction.

## EXPLORATION OF THE HERG CHANNEL INTERACTOME FOR A BETTER UNDERSTANDING OF INHERITED CARDIAC ARRHYTHMIAS

**Aline Rio**[1], Aurore GIRARDEAU[1], Gildas LOUSSOUARN[1], Nathalie GABORIT[1], Mikaël CROYAL[2], Céline MARIONNEAU[1], Michel DE WAARD[1], Isabelle BARO[1], Barbara RIBEIRO DE OLIVEIRA[1]
*[1] Institut du Thorax, Inserm UMR 1087 / CNRS UMR 6291, Nantes, France; [2] M.shark platform - Mass Spectrometry for Health, Analystical Research and Kinetics, Nantes Université, CHU Nantes, Inserm, CNRS, SFR Santé, Inserm UMS 016, CNRS UMS 3556, Nantes, France*

Long QT syndrome (SQTL), responsible for cardiac rhythm disorders that can lead to syncope or sudden death, is frequently associated with mutations in the KCNH2 gene encoding the hERG potassium channel. A nationwide initiative is currently phenotyping all 358 French hERG variants to assess their contribution to QTLS or short QT syndrome (SQTC). However, some variants do not directly affect channel function but may impair its interaction with partner proteins, making their pathogenicity difficult to interpret. This highlights the need for comprehensive understanding of the hERG protein interactome. This project aims to fill this gap by mapping the hERG channel interactome, identifying its functional partners and assess how pathogenic mutations these interactions. To address this, we first generated SPOT-tagged versions of the hERG channel by site-directed mutagenesis, inserting the tag at three different positions: the intracellular N- and C-terminal regions and the extracellular S1–S2 loop. These constructs were expressed in HEK 293 cells and analyzed electrophysiologically. The C-terminal tag was found to best preserve channel function and was selected for subsequent studies. We used CRISPR-Cas9 to insert the SPOT tag into the endogenous KCNH2 gene in human induced pluripotent stem cells (hiPSCs), enabling purification of the native hERG complex from hiPSC-derived cardiomyocytes (hiPSC-CMs). The first editing round produced 107 viable clones, currently being screened by PCR and sequencing to confirm successful tag insertion. In parallel, SPOT-tagged hERG was transiently expressed in hiPSC-derived ventricular cardioids, a model that recapitulates key features of human cardiac tissue. Immunoprecipitation of the tagged hERG protein using a SPOT-nanobody system (ChromoTek), followed by LC-MS/MS mass spectrometry, will allow identification of interacting proteins. These candidates will be validated through biochemical and electrophysiological analyses to assess their role in modulating wild-type and mutant hERG function. By characterizing the hERG interactome, this project will offer new insights into hERG channel physiology, improving interpretation of genetic variants and their role in QT syndromes. It may also uncover novel candidate genes involved in unexplained forms of LQTS.

Keywords: hERG channel; interactome; long QT syndrome; CRISPR-Cas9; proteomic; hiPS-CM; cardioid

## 16:00 - **Symposium 2: “Secondary ion channel dysfunction in patho-physiological processes”**

Organized by Vincent Seutin (Neurophysiology Lab, GIGA Neurosciences, Liège University, Belgium)

### NOVEL ASPECTS OF RENAL MAGNESIUM AND WATER TRANSPORT IN HEALTH AND DISEASE

**Joost Hoenderop**

*Radboudumc, Nijmegen, The Netherlands*

The kidney plays a pivotal role in maintaining mineral and fluid homeostasis, with magnesium emerging as an essential element for numerous structural and biochemical processes. Approximately 99% of filtered magnesium is reabsorbed along the nephron, highlighting the efficiency and importance of renal magnesium handling. Magnesium deficiency is clinically significant and may manifest as neuromuscular symptoms (e.g., cramps, fatigue, seizures) and cardiovascular disturbances such as arrhythmias. Advances in molecular physiology have shed light on the transport mechanisms underlying magnesium homeostasis. A key breakthrough has been the identification of members of the transient receptor potential (TRP) channel superfamily, particularly TRPM6 and TRPM7, which regulate divalent cation uptake in the kidney and intestine. In addition, regulatory pathways responding to mechanical stimuli, such as tubular flow, have been recognized as important modulators of electrolyte transport. Current research leverages a combination of epithelial cell models, transgenic animal studies, and emerging human-derived in vitro systems, such as kidney organoids and tubuloids. Tubuloids, derived from adult renal stem cells, comprise differentiated epithelial cells from various nephron segments and have proven capable of recapitulating physiological functions, including vasopressin-regulated water transport via aquaporin-2 water channels (AQP2) and vasopressin receptor-2 (V2R). These systems provide powerful platforms for dissecting the mechanisms of renal electrolyte and water transport under both healthy and pathological conditions. In this presentation, I will highlight recent discoveries in the molecular regulation of renal magnesium and water handling, with a focus on novel transport pathways and mechanosensitive elements. The talk will include a molecular "tour" along the nephron, emphasizing segment-specific contributions to overall mineral balance. Finally, I will discuss how kidney tubuloids are opening new avenues for disease modeling and the identification of pharmacological targets for disorders of electrolyte imbalance and water retention.

### HETEROGENEITY OF CARDIAC ION CHANNELS IN DISEASE – NEW INSIGHTS FOR ARRHYTHMIAS

**Karin R. Sipido**

*Dept Cardiovascular Sciences, KU Leuven, Leuven, Belgium*

Ventricular tachycardia that evolves to fibrillation and cardiac arrest remains a frequent cause of death after myocardial infarction, as well as in other pathologies that lead to heart failure consequent on a phenotypic remodeling of the cardiac tissue. Commonly used anti-arrhythmic therapy targets well-known ion channels that are at the backbone of the cardiac action potential, i.e. Na and Ca channels, and repolarizing K currents. Quite frequently however, implantable cardiac defibrillators are a first-choice therapy, because of limitations in pharmacotherapy. If a culprit arrhythmic site is identified within the heart, radiofrequency ablation is another approach. Avenues for the development of novel pharmacotherapy include: targeting of the ryanodine receptor (RyR) within ventricular myocytes, with the aim to reduce arrhythmogenic calcium waves; identification of ion channels and modulators that are unique to diseased tissue; increased use of tools that enhance translatability of experimental findings into clinical application. Considering heterogeneity at a multiscale level is essential in this development. In vivo, the cardiac remodeling process is heterogenous, and at cellular level, changes in ion channels and calcium handling, can vary within regions. The crosstalk between different (organ) systems and the heart can also be heterogeneous, e.g. the degree of innervation and consequently the functional modulation of ion channels. Other cell types can be connected to the cardiomyocytes, influencing their electrical activity, as exemplified by fibroblast-myocyte interactions. Tools to identify heterogeneity therefore need a multiscale approach from imaging and functional assessment in vivo, combining imaging and electrophysiology; tissue studies using live cardiac slices; isolated cardiomyocytes and fibroblasts, and molecular approaches using transcriptomics and imaging. The above concepts will be illustrated by recent and ongoing studies on arrhythmia mechanisms in ischemic cardiomyopathy.

**DENDRITIC MECHANISMS OF COGNITIVE COMORBIDITY IN EPILEPSY**

**Heinz Beck**
*Institute of Experimental Epyleptology and Cognition Research, Bonn, Germany*

The main symptom of epilepsies is unpredictable seizures, and efforts to develop novel treatments for epilepsies are almost exclusively focused on controlling seizures. However, in addition to seizures, many epilepsies including temporal lobe epilepsy show cognitive deficits as a clinically relevant comorbidity. Cognitive comorbidities are seen in 60-70% of epilepsy patients, and profoundly impact the patients' quality of life. While controlling seizures improves cognition in many cases, clinical evidence indicates that there are additional causes for cognitive dysfunction. Dendritic spikes are a cornerstone of dendritic integration that enable neurons to generate precise action potential outputs to temporally and spatially clustered inputs. They are therefore important in mediating sharply tuned neuronal responses to features of specific populations of input neurons, and have been strongly implicated in place-related firing and spatial navigation. Using two-photon glutamate uncaging in-vitro, we show that dendritic integration via dendritic spikes is strongly altered in chronic epilepsy, with a marked degradation in the capability of hippocampal dendrites to perform input feature detection. In-vivo two-photon imaging revealed that this deficit in dendritic integration is associated with altered place-related firing of CA1 neurons. A selective sodium channel blocker reversed the deficits in dendritic integration and place coding, and reverses hippocampal memory deficits. Thus, a dendritic channelopathy may underlie cognitive deficits in epilepsy and targeting it pharmacologically may constitute a new avenue to enhance cognition.

**DO CLC-2 CHLORIDE CHANNELS PLAY A ROLE IN HUMAN PANCREATIC DUCTAL ADENOCARCINOMA?**

Concetta Altamura, Ilaria Saltarella, Paola Laghetti, Simone Dell'Atti, Giulia Maria Camerino, Maria Raffaella Greco, Maria Antonia Frassanito, Rosa Angela Cardone, **Jean-François Desaphy**

*Section of Pharmacology, Department of Precision and Regenerative Medicine, University of Bari Aldo Moro, Italy*

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers. Despite recent progress of combined chemotherapy, new therapies are urgently needed. Ion channels have been pinpointed as potential regulators of tumor cell proliferation, invasiveness, and chemotherapy resistance. We investigated the role of ClC chloride channels in human pancreatic adenocarcinoma cell lines (Panc-1, Mia-PACA), cancer stem cells (CSC) derived from Panc-1, and healthy pancreatic cell line (HPDE). Using RT-PCR, we found a greater transcript expression of ClC-2 and ClC-7 chloride channels and their respective auxiliary subunits HEPACAM and Ostm1 in tumoral cell lines compared to HPDE. Overexpression of ClC-2 protein was confirmed by WB in cancer cell lines. Using patch-clamp, whole-cell chloride currents resembling ClC-2 were recorded in ~25 % of patched Panc-1 cells and CSC (n=55 each), whereas no chloride current was found in HPDE cells (n=22). These currents were blocked by meclofenamic acid (MCFA), a well-known ClC-2 inhibitor (inhibition >50 % at 30 µM). Using flow cytofluorometry, we observed that 3 days incubation of CSC with MCFA reduced cell proliferation and increased cell apoptosis. These effects were low but significant with 30 µM MCFA and very marked at higher MCFA concentrations (100-300 µM). Running Kaplan-Meier plotter on 150 PDAC patients, we observed that high levels of ClC-2 expression were associated with a shorter Disease Free Survival. These findings strongly suggest ClC-2 channel as a negative prognostic marker in PDAC, identifying it as a potential therapeutic target for this devastating disease. Supported by Italian Ministry of University (PRIN n. 20174TB8KW-002).

## 17:45 - **Oral communication session 2 : "Ion channel modulation: from molecule identification to physiological effects"**

Organized by Alban Girault (LPCM UR UPJV4667, Université de Picardie Jules Verne, Amiens, France)

### SCREENING AND OPTIMIZATION OF CELL-PENETRATING PEPTIDES FOR DELIVERY IN HUMAN CARDIOMYOCYTES

**Serena Hachem**[1], Flavien Charpentier[1], Michel De Waard[1]
*L'Institut du thorax, Université de Nantes, France*

*[1] Nantes Université, CNRS, INSERM, L'institut du thorax, F-44000 Nantes, France, [2] LabEx "Ion Channels, Science & Therapeutics", F-06560 Valbonne, France.*

Cardiac arrhythmias are often linked to abnormal expression or function of voltage-gated sodium channels, particularly Nav1.5, encoded by the SCN5A gene, a key determinant of action potential initiation and propagation in cardiomyocytes. Mutations and dysregulations of SCN5A gene often lead to severe cardiac arrhythmias, including Brugada syndrome. However, many therapeutic candidates face critical delivery challenges, particularly in cardiac cells. In this context, Cell-Penetrating Peptides (CPPs) have emerged as a powerful class of short peptides capable of transporting various cargo molecules across cellular membranes, including proteins, nucleic acids, and small drugs. Their favorable physico-chemical properties and non-viral nature make them attractive candidates for therapeutic delivery, especially in hard to transfect cells such as cardiomyocytes. Despite their potential, CPP efficiency varies widely depending on sequence, structure, and cellular context, underscoring the need for careful screening and optimization. This project aims to evaluate and to assess the uptake, localization and distribution of a panel of CPPs in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), a physiologically relevant in vitro model. For this, ten fluorescently labeled CPPs were tested across 3 different cell lines: AC16 (immortalized human ventricular cardiomyocytes), isolated neonatal mouse cardiomyocytes, and hiPSC-CMs. Cellular uptake and subcellular distribution were first evaluated qualitatively using fluorescence microscopy. To assess uptake efficiency more quantitatively, fluorescence signal was measured using a plate reader, on both AC16 and hiPSC-CMs cells. To date, we have demonstrated that several of the tested CPPs can successfully enter cardiomyocytes via different mechanisms. Our preliminary data enabled us to identify a subset of peptides with favorable uptake profiles. This analysis contributes to improving the delivery efficiency of CPP, and allows us to select efficient CPPs as promising candidates and for subsequent conjugation to cargo molecule of choice. Looking forward, the selected CPP will serve as a delivery vector for promising tools for gene regulation. Among the considered molecules, Peptide Nucleic Acids (PNAs) emerged as new potential transcriptional modulators of the SCN5A gene. Our study supports and lays the foundation for future gene therapies strategies based on CPP-

### COMPREHENSIVE IN VITRO EVALUATION OF FIVE NAV BLOCKERS IN TWO MUTANTS RESPONSIBLE FOR PARAMYOTONIA CONGENITA

**Nurcan Inci[1],** Amandine Ligot[1], François C. Wang[1,2], Samira Abdou Ide[1,2], Bernard Lakaye[3], Vincent Seutin[1]

[1] *Laboratory of Neurophysiology, Giga Neurosciences University of Liège, Belgium;* [2] *Clinical Neurophysiology, Department of Physical Medicine and Readaptation, Liège University Hospital, Belgium;* [3] *Laboratory of Molecular Regulation of Neurogenesis, GIGA Neurosciences, University of Liège, Belgium*

Paramyotonia congenita is a rare neuromuscular disease caused by mutations in the Nav1.4 channel, which is the voltage-gated sodium channel expressed by skeletal muscle cells. Patients with this disease exhibit various symptoms, including delayed muscle relaxation, cold-aggravated painful contractions, and hyperexcitability. Current treatments for these patients involve sodium channel blockers. The most common drug offered to the patients is mexiletine, which has cardiovascular side effects and does not have the same efficacy across the mutations. In this study, we focused on two specific mutations: L1436P, which we observed in multiple patients from Liège, Belgium, and R1448H, a common mutation found in the channel's voltage-sensing region. Since Nav channels have three different functional states (closed, inactivated, open) we conducted a comprehensive pharmacological analysis using various sodium channel blockers with different mechanisms of action: lamotrigine (an anticonvulsant that inhibits repetitive firing), lidocaine (a fast-acting local anaesthetic that blocks sodium channels in their inactivated states), flecainide (a class IC antiarrhythmic with preferential open-channel block), ranolazine (an antianginal agent that enhances slow inactivation), and lacosamide (an anticonvulsant that enhances slow inactivation as well). The effect of the drugs on wild-type and mutant Nav1.4 channels was tested to find the most suitable drug for different mutations while minimizing effects on wild-type channels, given the heterozygous nature of the disease. All experiments were performed using stably transfected HEK293 cell lines expressing WT channels or Nav1.4 channel mutants, and the drug responses were quantified using whole-cell patch clamping. Our analysis confirmed that the blocking potency of the drugs varies for wild-type channels when tested in different states. Moreover, confirming our hypothesis, we demonstrated that drug efficacy differs between mutant and wild-type channels for some drugs. These findings suggest that personalized treatment approaches may become a reality in the near future.

## CHOLESTEROL AS A MODULATOR OF THE IKS CHANNEL COMPLEX

**Frans Lisse[1]**, Dieter van de Sande[2], Dirk J. Snyders[2], Alain J. Labro[1]
[1] *Department of Basic and Applied Medical Sciences, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium;* [2] *Department of Biomedical Sciences, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, 2610 Antwerp, Belgium*

The slow delayed rectifier potassium current (IKs), mediated by the KCNQ1+KCNE1 channel complex, plays a crucial role in cardiac action potential repolarization, where it is accepted to form a repolarization reserve. The IKs current increases upon adrenergic (sympathetic) stimulation, through i.e., the activation of protein kinase A (PKA). To evaluate the direct and indirect effects of acute cholesterol modulation on the IKs current, CHO cells expressing the KCNQ1 and KCNE1 subunits were exposed to 5mM methyl-β-cyclodextrin (MβCD), which, after 20 minutes, resulted in a 25% reduction in the plasma membrane cholesterol content.

Cholesterol depletion increased the IKs-like current amplitude by approximately 20%. Interestingly, the affinity to the antiarrhythmic drug quinidine increased, as reflected by a significantly decreased IC50 value (22 µM) compared to the control (61 µM). As cholesterol content defines cell membrane fluidity and lipid raft integrity, insight into the dynamics of the KCNQ1+KCNE1 channel complex was gained by performing a split NanoLuciferase complementation assay. The luminescent signal increased within 20 min of MβCD administration, indicating an increase in the likelihood of interaction between KCNQ1 and KCNE1 subunits. This might be caused by more subunits interacting or the closer proximity of both subunits. These preliminary data suggest that assembly of the KCNQ1+KCNE1 channel complex at the plasma membrane is a cholesterol-sensitive process whereby acute cholesterol depletion increases the IKs-like current amplitude in CHO-cells.

## BENEFICIAL APD-SHORTENING EFFECTS, BUT DELETERIOUS NEGATIVE INOTROPISM OF IKS-ACTIVATOR DOCOSAHEXAENOYL GLYCINE (DHA-GLY) IN LONG QT SYNDROME TYPE 2

**Julien Louradour[1]**, Tibor Hornyik[1], Alicia De la Cruz[2], Irene Hiniesto-Iñigo[2], Nicolò Alerni[1], Miriam Barbieri[1], Lluís Matas[1], Sara I. Liin[2], H. Peter Larsson[2], Katja Odening[1]

*[1] Translational Cardiology, Institute of Physiology and Department of Cardiology, University of Bern, Switzerland; [2] Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden*

*Background and aim:* Loss-of-function mutations in KCNQ1 and KCNH2 (α-subunits of the IKs and IKr-conducting repolarising $K^+$ channels) lead to long QT syndrome type 1 (LQT1) and 2 (LQT2), respectively. These channelopathies present with longer action potential duration (APD) and prolonged QT interval on ECG, which can ultimately lead to deadly arrhythmias. While beta-blockers or implantable cardioverter defibrillators are used to reduce subsequent arrhythmias in long QT patients, there are no current treatments that would target the cause, i.e. the prolonged cardiac APD. Here we investigated the therapeutic potential of the polyunsaturated fatty acid docosahexaenoyl glycine (DHA-gly) in normalizing APD and QT interval in LQT2 by increasing IKs.

*Methods:* The effects of DHA-gly on electrical and mechanical parameters were assessed in Xenopus laevis oocytes, wild-type (WT), LQT1 (KCNQ1-Y315S) and LQT2 (KCNH2-G628S) transgenic rabbit models, and human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs).

*Results:* DHA-gly increased IKs in oocytes and WT ventricular cardiomyocytes (VCMs) in a dose-dependent manner. Consequently, DHA-gly shortened cellular APD and QT interval ex vivo in WT (APD90: 316.3 ± 16.5 to 280.9 ± 22.4 ms, QT: 215.2 ± 2.6 to 206.6 ± 2.8 ms) and LQT2 rabbits (APD90: 500.4 ± 16.1 to 408.6 ± 38.7 ms, QT: 253.5 ± 1.8 to 242.8 ± 8.3 ms), but not in LQT1 (APD90: 434.5 ± 24.7 to 417.5 ± 23.9 ms, QT: 236.1 ± 8.3 to 230.1 ± 12.1 ms). However, DHA-gly was unable to reduce arrhythmia formation in LQT2. Beneficial APD/QT shortening effects were accompanied by a detrimental decrease in both cellular and ventricular contraction across all genotypes, including LQT1, which could be due to a shortening in $Ca^{2+}$ transient duration observed in VCMs and hiPSC-CMs.

*Conclusions:* DHA-gly-induced IKs enhancement shows promising results in shortening APD/QT in LQT2 rabbits, while having no effect on LQT1 (impaired IKs). However, its adverse effect on cardiac contractility, even in LQT1, makes it unsuitable to treat LQTS patients. This study highlights the importance of considering both electrical and mechanical effects of potential therapeutic compounds prior to clinical translation.

# Tuesday, September 16th 2025

## 08:30 - **Symposium 3: "Ion signaling and cell bioenergetics in cancer"**

Organized by Valerio Farfariello (Laboratoire PhyCell - INSERM UMR1003, Lille, France)

### MULTIPLE ROLES OF MITOCHONDRIAL ION CHANNELS IN POOR PROGNOSIS CANCERS

Veronica Carpanese[1], Beatrice Angi[1], Carlotta Paoli[1], Valentina Brillo[1], Erich Gulbins[2], Vanessa Checchetto[1], Tatiana Varanita[1], Lucia Biasutto[3], Andrea Mattarei[4], **Ildiko Szabo[1]**

*[1] Department of Biology, University of Padova, Italy; [2] Department of Molecular Biology, University of Duisburg-Essen, Germany; [3] CNR Institute of Neurosciences, University of Padova, Italy; [4] Department of Pharmaceutical Sciences, University of Padova, Italy*

Mitochondrial ion channels affect cell fate, as they importantly regulate bioenergetic efficiency but also contribute to apoptosis execution. We have developed a number of mitochondria-targeted drugs against mitochondrial potassium channels (Kv1.3, KCa3.1, TASK-3) and provided evidence that some of these drugs exert a strong tumor-reducing effect in vivo, even for poor prognosis cancers such as pancreatic ductal adenocarcinoma. Likewise, genetic manipulation of the expression level of these channels both in the cancer cells (Paoli et al, in preparation, Carpanese et al, in preparation) and in the immune cells (Angi et al, in preparation) of the tumor microenvironment affect tumor progression and metastatic spread in vivo. We have furthermore obtained information about the intact cell interactome of Kv1.3 and KCa3.1, both showing dual localization to the plasma membrane and to mitochondria, revealing a number of unexpected interaction partners that strongly modulate cancer-relevant signaling (Prosdocimi, Carpanese et al, 2024, Carpanese et al, 2025, in press). Overall, mitochondrial ion channels are emerging as important pharmacological targets against poor prognosis cancers due to their crucial roles in various cancer-related signaling processes and in modulating mitochondrial function.

### BAPTA, AN INTRACELLULAR CALCIUM CHELATOR, AND ITS IMPACT ON METABOLISM & CELL DEATH IN B-CELL CANCERS: SADLY, NOT AT ALL A STORY ABOUT CALCIUM

Femke Speelman-Rooms[1], Roberto Ornelas Guevara[2], Kato Huysmans[1], Olivier Romito[3], Wout Balcaen[1], Flore Sneyers[1], Maria Manifava[4], Thomas Voets[1], Steven Verhelst[1], Mohamed Trebak[3], Martin Bootman[5], Nicholas Ktistakis[4], **Geert Bultynck[1]**

*[1] KU Leuven, Belgium; [2] ULB, Brussels, Belgium; [3] Pittsburgh University, PA, USA; [4] Babraham Institute, Cambridge, UK; [5] The Open University, Milton Keynes, UK*

Intracellular $Ca^{2+}$ chelators, such as BAPTA, are widely used to identify the role of $Ca^{2+}$ signaling in cellular processes underlying physiology and pathogenesis. However, accumulating evidence shows that BAPTA directly affects intracellular targets, thereby evoking cellular effects independently of its $Ca^{2+}$-chelating properties. Key to this conclusion has been the use of TF-BAPTA, a BAPTA analogue with very low $Ca^{2+}$-binding affinity ($K_D$ ~65 μM; compared to 160 nM for BAPTA). If similar results are obtained with BAPTA and TF-BAPTA, the observed effects are considered $Ca^{2+}$ independent since we validated that TF- BAPTA is not able to chelate $Ca^{2+}$ in the cytosol, microdomains and nanodomains.

In cells, BAPTA directly inhibited PFKFB3, a key glycolytic enzyme producing fructose 2,6-bisphosphate, a positive modulator of PFK-1 activity. By inhibiting PFKFB3, BAPTA (and TF-BAPTA) resulted in profound inhibition of mTORC1 activity, thereby evoking downregulation of the short-lived anti-apoptotic protein, MCL-1. In MCL-1-dependent B-cell cancer cells, this resulted in apoptosis that could be rescued by introducing a non-degradable MCL-1 mutant.

In further work, we examined whether the effects of BAPTA could be mimicked by using selective PFKFB3 inhibitors, such as KAN0438757. We found that KAN0438757 decreased mTORC1 activity, downregulated MCL-1 protein levels and boosted Venetoclax-induced cell death in MCL-1 dependent cancer cells.

Next, we explored the impact of BAPTA on autophagy. Surprisingly, we found that, despite inhibiting mTORC1 activity, BAPTA (and TF-BAPTA) suppressed, not stimulated, autophagic flux. By examining the impact of BAPTA (and TF-BAPTA) on the autophagic pathway in basal conditions and upon mTORC1 inhibition (e.g. using PP242), we found that it blocked autophagy downstream of the ULK1 initiation complex.

Overall, our work shows that BAPTA evokes profound and rapid cellular alterations towards metabolism, autophagy and cell death, irrespective of its $Ca^{2+}$-chelating properties.

## CALCIUM AND REDOX SIGNALS AT CELLULAR INTERFACES: IMPLICATIONS FOR IMMUNITY AND CANCER


**Ivan Bogeski**

*University Medical Center, Göttingen, Germany*


Calcium ($Ca^{2+}$) signals at subcellular contact sites, such as endoplasmic reticulum (ER)–plasma membrane and ER–mitochondria interfaces, coordinate essential processes including gene transcription, metabolism and cell death. Our recent findings highlight the critical role of redox-dependent modulation of calcium transport systems at these sites in both immune regulation and cancer progression. We have demonstrated that oxidative stress alters store-operated $Ca^{2+}$ entry (SOCE) through post-translational modification of Orai1 and STIM2, suppressing $Ca^{2+}$ influx and affecting downstream signaling in T-cells and melanoma cells. Redox-sensitive control of $Ca^{2+}$ transfer at organelle junctions was further explored using genetically encoded biosensors and chemogenetic tools, revealing compartment-specific $Ca^{2+}/H_2O_2$ crosstalk that impacts mitochondrial function and cell migration.

In T cells, we demonstrated that mitochondrial $Ca^{2+}$ uptake via the calcium uniporter (MCU) complex regulates cytokine production and metabolic adaptation, linking organellar $Ca^{2+}$ handling to immune effector function. In parallel, proteomic profiling of natural killer (NK) cell-mediated cytotoxicity identified $Ca^{2+}$-responsive signatures predictive of melanoma responsiveness to immune checkpoint therapy.

Together, these studies define a unifying framework in which redox-modulated $Ca^{2+}$ signaling at cellular interfaces orchestrates immune cell behavior and tumor cell fate. In this talk, I will highlight these key mechanisms and present novel, unpublished data that further advance our understanding of $Ca^{2+}$ and redox-controlled signaling in immunity and cancer.

**COLD-ACTIVATED SPINAL CIRCUITRY AND TRP CHANNEL CONTRIBUTION IN CHEMOTHERAPY INDUCED PERIPHERAL NEUROPATHY (CIPN)**

**Marina Villaro**, Félix Viana, Ana Gomis
*Instituto de Neurociencias, UMH-CSIC, Sant Joan d'Alacant, Spain*

Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent and disabling side effect of neurotoxic anticancer agents, characterized by peripheral nerve damage. Among its painful symptoms, cold hypersensitivity is particularly severe, often leading to dose reduction or treatment discontinuation, with serious implications for patient survival and well-being. While peripheral mechanisms—such as ion channel dysregulation in primary afferent neurons—have been extensively studied, much less is known about how these changes affect central processing at the spinal cord level. This project investigates cold-evoked neuronal activation patterns in the dorsal horn, using a mouse model of CIPN induced by oxaliplatin. Neuronal activation was permanently tagged in TRAP2-iCreERT2-tdTomato mice during exposure to innocuous and noxious cold stimuli. A second stimulation under identical conditions was performed to determine the temporal stability of cold-induced neuronal activation, assessed by cFos staining. Additionally, to investigate the contribution of cold-sensitive ion channels, naïve mice received intraplantar injections of AITC (a TRPA1 agonist) or WS12 (a TRPM8 agonist), followed by analysis of spinal neurons activated by noxious cold. Behavioral assays confirmed the development of cold hypersensitivity in CIPN mice. At the spinal level, oxaliplatin-treated animals showed increased numbers of tdTomato+ neurons following cold stimulation, with a higher degree of co-localization with cFos after a second noxious cold exposure. Interestingly, cFos labelling alone did not differ significantly between conditions, suggesting possible limitations in sensitivity or temporal dynamics between markers. Pharmacological activation of TRPA1 and TRPM8 nerve endings in naïve mice revealed that both agonists recruit comparable numbers of dorsal horn neurons, with partial overlap with the neuronal populations activated by noxious cold in both cases. These results suggest that both channels contribute to spinal cold processing but do not fully define the cold-responsive neuronal ensemble. Overall, these findings reveal stat-dependent changes and cold-evoked spinal circuit activation during CIPN and highlight the value of TRAP2-based activity tagging for studying persistent circuit alterations. Further studies, integrating molecular profiling and electrophysiological approaches, will be essential to identify specific neuronal subpopulations involved in cold hypersensitivity.

Keywords: oxaliplatin, dorsal horn, cold hypersensitivity, TRPA1, TRPM8, neuron labelling.

## 17:15 - **Symposium 4: "Ion channels in plants and fungi"**

Organized by Jean-Sébastien Rougier (Institute of Biochemistry and Molecular Medicine, Berne, Suisse)

### IDENTIFICATION OF THE $CO_2$ SENSOR AND GUARD CELL SIGNAL TRANSDUCTION MECHANISMS THAT CONTROL STOMATAL MOVEMENTS

**Julian Schroeder**

*School of Biological Sciences, Department of Cell and Developmental Biology & Department of Bioengineering, University California San Diego, La Jolla, USA*

Due to the rising atmospheric $CO_2$ concentration, $CO_2$ control of stomatal conductance and plant transpiration will affect global plant water loss, water use efficiency, leaf heat stress and optimal stomatal conductance of plants. Elevated $CO_2$ concentrations in leaves cause stomatal closure, whereas reduced $CO_2$ concentrations result in stomatal opening. The continuing rise in the atmospheric $CO_2$ concentration is narrowing stomatal pores globally via $CO_2$ enhanced activation of anion channels and regulation of other ion transporters and channels. Genetic mutants in MAP kinases (MPK12 and 4), Raf-like kinases (HT1, CBC1 and 2) and carbonic anhydrases (bCA4 and 1) have been identified that disrupt or impair $CO_2$ signal transduction. However, the molecular signal transduction mechanisms that constitute the primary $CO_2$ sensor were unknown. We have found that high $CO_2$ /bicarbonate directly induces interaction of MAP kinases MPK12 and MPK4 with the HT1 protein kinase in vitro and in planta. At low $CO_2$, MPK4 and MPK12show reduced interaction with the HT1 kinase enabling HT1 activation. Activated HT1 phosphorylates and thus activates the Raf-like protein kinases Convergence of Blue Light and $CO_2$ (CBC1) at critical residues resulting in low $CO_2$-induced stomatal opening, which involves H pump activation and anion channel downregulation. Interestingly and unexpectedly, MAP kinase activity is not required for $CO_2$ sensor function and $CO_2$-triggered HT1 inhibition in vitro and high $CO_2$-induced stomatal closing in planta. Structural AlphaFold2- and Gaussian-accelerated dynamics-generated modelling together with stomatal response phenotypes of genetic mutants provide insights into how specific mutant alleles disrupt $CO_2$ sensor function: The known dominant HT1 point mutants, ht1-R102K and ht1-A109V, and a computationally predicted MPK12 mutant greatly reduce the $CO_2$ induced MPK12-HT1interaction and maintain HT1 kinase activity thus providing a model for how they disrupt $CO_2$ signaling. Our independent unbiased forward genetic screen for stomatal insensitivity to high $CO_2$ in the grass species, Brachypodium distachyon, has led to isolation of a mutant in the closest MAP kinase homolog to the $CO_2$ signaling Arabidopsis MAP kinases. Further analyses of the Brachypodium MAP kinase suggest an analogous mechanism for stomatal $CO_2$ sensing. The presented findings reveal biochemical, molecular genetics, physiological and structural modeling bases for the long-sought primary stomatal $CO_2$ sensor and the immediate downstream signaling mechanism in plants.

### STRUCTURE AND FUNCTION OF PLANT ION CHANNELS

**Alexis De Angeli[1]**, Elsa Demes[1], Sangho Lee[2], Yeongmok Lee[2]
*[1] Institut des Sciences des Plantes de Montpellier, CNRS, Montpellier, France; [2] Sungkyunkwan University, South Korea*

In plants, ion transport systems play essential functions for cellular responses to the environment. An important example of this role is the regulation of leaf transpiration through the stomata pore, a process involved in drought tolerance. The regulation of the stomata pore aperture depends on molecular properties of the ion transporters and channels. Several classes of ion channels are present in stomata, and among them, we focused on the plant-specific Aluminum-activated malate transporters (ALMTs) family. This family is involved in crucial physiological functions of land plants and, in Arabidopsis thaliana, its members are localized at the plasma or vacuolar membrane. The vacuolar ALMTs mediate malate and chloride transport regulating stomata aperture and in the regulation of the plant cell metabolism. This family of membrane proteins presents a unique folding with specific biophysical properties. We used a combination of approaches to link the structural and functional characteristics of vacuolar ALMTs with their functions in vivo.

### GATING AND REGULATION OF FUNGAL POTASSIUM CHANNELS

**Anthony Lewis**
*University of Portsmouth, UK*

Fungal pathogens are a major cause of life-threatening infections and a significant threat to crop yield and global food security. Fungal genomes encode a unique plasma membrane potassium channel, TOK (Two-pore Outwardly-rectifying K+ channel), which has no known structural or functional homologues in mammals or plants. TOK channel subunits are distinctive in topology with an 8TM/2P structure and unique in their strict outwardly-rectifying behaviour above the reversal potential. Here, we present the identification, biophysical characterization and regulation of the Candida albicans TOK homologue (CaTOK). First, we reveal conserved amino acids lining the conduction pathway represent key sites governing outward rectification. We propose a simple mechanistic model whereby the reversal potential determines ion occupancy, and thus conductivity, of the selectivity filter gate. Secondly, we demonstrate that CaTOK is a pH-sensitive channel, and can be modulated by many other environmental cues that govern morphological transitions associated with fungal virulence. Our goal is to move towards validating TOK channels as viable, therapeutic targets for combating deleterious fungal infections in humans and plants.

### VACUOLAR MALATE AND FUMARATE TRANSPORT THROUGH ATALMTS

**Elsa Demes-Causse**, Roxane Doireau, Paloma Cubero-Font, Alexis De Angeli
*Institut des Sciences des Plantes de Montpellier, Montpellier, France*

Plants fix a proportion of the carbon into organic acids (i.e. malate and fumarate). These organic acids are then used in many metabolic pathways (i.e. TCA cycle, regulation of cytosolic pH, osmotic pressure) and have a role in stomata movements too. Therefore, the storage of organic acids has to be tightly regulated. In this study, we focus on one ion transporter family for which some members are able to transport organic acids. The Aluminum-activated malate transporters (ALMTs) family is involved in crucial physiological functions of land plants. Arabidopsis thaliana counts 14 members, some localized at the plasma membrane and others at the vacuolar membrane. Here we analyzed single or multiple knock out plants for the main vacuolar AtALMTs (AtALMT9, AtALMT5 and AtALMT4) which are known to transport malate and/or fumarate into the vacuole. Some of the multiple knock out plants show severe growth defects with early senescent leaves. Therefore, we wanted to investigate the transport of malate and fumarate in plants lacking one or more vacuolar AtALMTs. By using the electrophysiological patch clamp technique on Arabidopsis thaliana vacuoles from each mutant, we analyzed the transport capacity of malate and fumarate in each of the mutants. When all three of the vacuolar transporters are knocked out, the transport of fumarate is almost completely impaired. This study shows that the vacuolar AtALMT channels are crucial for the regulation of organic acid accumulation in the vacuole.

# Wednesday, September 17th 2025

## 09:30 – Symposium 5: "Genetically encoded pharmacology in ligand-gated ion channels"

Organized by Laetitia Mony (Institute of Biology of Ecole Normale Supérieure, Paris, France)

### NATIVE NMDA RECEPTORS IN THE BRAIN: FROM ATOMIC STRUCTURE TO SYNAPTIC PHYSIOLOGY

**Shujia ZHU,** Ming Zhang, Xuejing Huang, Xiaole Sun
*Southern University of Science and Technology, Shenzhen, China*

The cerebral cortex and hippocampus are critical brain regions for learning and memory, processes that rely on activity-induced synaptic plasticity mediated by N-methyl-ᴅ-aspartate receptors (NMDARs). However, the subunit assembly and molecular architecture of these endogenous NMDARs (eNMDARs) remain poorly understood. Using conformation- and subunit-specific antibodies, we purified eNMDARs from adult rat cerebral cortex and hippocampus and resolved three major eNMDAR subtypes of GluN1-N2A-N2B, GluN1-N2B, and GluN1-N2A receptors, at resolutions up to 4.2 Å. These subtypes exhibited a particle ratio of 9:7:4, indicating that approximately half of GluN2A and GluN2B subunits are incorporated into tri-heterotetrameric complexes (Cell 2025). Our findings reveal the structural and functional complexity of eNMDARs and shed light on structure-based therapeutic design targeting these receptors in vivo. In the second part, we present the structural basis for the diverse regulatory roles of magnesium ($Mg^{2+}$) in NMDAR function. $Mg^{2+}$ is a key ion that enables NMDARs to act as coincidence detectors in excitatory synaptic transmission. Cryo-EM structures revealed three distinct $Mg^{2+}$ binding sites on the GluN1-N2B receptors. Site I, located at the selectivity filter, features an asparagine ring that coordinates $Mg^{2+}$ and mediates voltage-dependent $Mg^{2+}$ block. Site II and III reside in the N-terminal domain (NTD) of GluN2B subunit, mediating GluN2B-specific allosteric potentiation and NTD-driven allosteric inhibition, respectively (Neuron 2025). These findings elucidate the multifaceted mechanisms by which $Mg^{2+}$ regulates NMDAR function and synaptic plasticity.

### GENETICALLY-ENCODED TOXINS ACTING ON Kv CHANNELS AND NMDA RECEPTORS

Asaf Bittan, Ido Carmi, Shaden Zoabi, Michael Andreyanbov, **Shai Berlin**
*Technion, Haifa, Israel*

NMDA-type glutamate receptors (NMDARs) play essential roles in neuronal development, synaptic plasticity, and cell survival. They are also critically implicated in a wide range of neurological and psychiatric conditions. Despite their importance, current pharmacological strategies to modulate NMDARs face major limitations, including insufficient subtype selectivity and lack of spatial specificity. Genetic approaches, particularly those involving genetically encoded peptide modulators (such as those derived from animal venoms), offer a promising path forward. Yet, while venom-derived peptides targeting other ion channels are increasingly being identified, NMDAR-selective toxins remain exceedingly rare with only two natural families known to date. In this work, we present two complementary genetically encoded approaches for targeting NMDARs. The first is a soluble and secreted peptide derived from conantokin toxins from Conus snails, denoted naked Con P (Carmi et al., PNASnexus, 2025). Unexpectedly, though naked Con-P maintains its ability to inhibit GluN2B-containing receptors as the parent toxin, the engineered variant uniquely acquired the ability to also potentiate GluN2A-containing synaptic receptors. The second tool is t-PnTx, a membrane-tethered spider toxin engineered from Phoneutria nigriventer (unpublished). This tool builds on our prior development of metaPoda and metaPhix, namely membrane-tethered spider toxins that potently target Kv4 channels (Andreyanov et al., J. Neurosci., 2024), for spatially-confined, postsynaptic inhibition of NMDARs. Together, these two systems offer a modular toolkit: secreted constructs for circuit-level NMDAR suppression, and membrane-tethered toxins for precise, cell- and synapse-specific modulation. This work represents a significant step forward in our ability to probe and manipulate NMDAR signaling with high molecular, spatial, and temporal precision. Importantly, these tools also lay the foundation for next-generation therapeutic strategies aimed at treating NMDAR-related disorders such as epilepsy, neurodevelopmental disorders, and schizophrenia.

### MECHANISMS OF NANOBODY MODULATION OF A PENTAMERIC CHANNEL-RECEPTOR

Nathalie Barilone[1], Maria Vangelatou[1], F. Zahra Marouf[1], Pierre Lafaye[2], Gabriel Aymé[2], Pierre-Jean Corringer[1], **Marie Prévost[1]**
*[1] Institut Pasteur, Université Paris Cité, CNRS UMR 3571, Signaling and Receptors Dynamics Unit, Paris, France; [2] Institut Pasteur, Université Paris Cité, CNRS UMR 3528, Antibody Engineering platform, Paris, France*

Nicotinic acetylcholine receptors are essential pentameric-channel receptors in the nervous system. Their dysfunction is linked to severe psychiatric and neurological diseases such as nicotine addiction, schizophrenia or Alzheimer's disease. They bind neurotransmitters at the synapse, triggering the opening of their ion channel, in turn generating neuronal electrical response. The conformational changes associated with their activity are precisely regulated according to a 3-states allosteric model and are highly sensitive to pharmacological ligands, such as anesthetics, nicotine, alcohol and even small proteins. I will present an example of such modulation with our recent work, where we investigated by cryo-electron microscopy the potentiation mechanism of the human alpha7 nicotinic acetylcholine receptor by nanobodies. This structure-function analysis provides new pharmacological insights on the modulation of those receptors.

### MAKING AN AMPA RECEPTOR SLOW

**Jennifer D. Noonan,** Irene Riva, Andrew J. R. Plested
*Institut für Biologie, Humboldt Universität zu Berlin, Germany*

AMPA receptors are known for their fast kinetics. But recently AMPA receptors displaying slow currents that decay in 500 ms and are resistant to desensitization have been reported in brain slices. At the synapse, receptors are in complex with auxiliary proteins that modulate receptor kinetics and likely underpin the slow AMPA response. However, it is not known what specific subunits or auxiliary proteins contribute, in particular because no known combination of subunits produces such slow kinetics (about 500x slower than the usual decay). By utilizing a chimeric TARP of gamma-2 and gamma-8 (termed super TARP) that exerts super-active effects on the AMPA receptor, we observed a subunit-dependent modulation of GluA2(R):GluA1 heteromers. The superTARP specifically slowed kinetics of GluA2(R) homomeric receptors, but not GluA1. In the GluA2(R): GluA1 heteromer, the superTARP again slowed receptors kinetics, confirming that not only do TARPs exhibit subunit-dependent modulation of receptor function, but also indicating a dominant role for GluA2 in the heteromer. The super TARP also introduced polyamine block in heteromeric receptors. However, the responses generated were not slow enough to account for slow AMPA currents in neurons. In an effort to identify additional subunit combinations that produce slow AMPA responses, we turned to published RNAseq data from the hippocampus and tested subunits with heterogeneous expression patterns in hippocampal pyramidal neurons. Surprisingly, the "fast" AMPA subunit GluA4, presumed absent from pyramidal neurons, in combination with various auxiliary proteins showed the slowest decays measured to date. This work highlights new types of AMPA receptor diversity and identifies novel potential mediators of the slow AMPA response.

# POSTER ABSTRACTS

## P1 - CHARACTERIZATION OF A MOUSE MODEL CARRYING THE CAV3.1 RECURRENT MUTATION FOUND IN THE NEURODEVELOPMENTAL SCA42ND DISEASE

**Theo Petrizzelli[1],** Clément Marchal[1], Claire Bernat[1], Vincent Cantagrel[2], Philippe Isope[3], Sophie Nicole[1], Philippe Lory[1]
*[1] Institut de Génomique Fonctionnelle, Montpellier, France; 2 Institut Imagine, Paris, France; [3] Institut des Neurosciences Cellulaires et Intégratives, Strasbourg, France*

Characterization of a mouse model carrying the Cav3.1 recurrent mutation found in the neurodevelopmental SCA42ND disease Theo Petrizzelli1,2, Clément Marchal1,2, Claire Bernat1,2, Vincent Cantagrel3, Philippe Isope4, Sophie Nicole1,2 and Philippe Lory1,2 1IGF, Université de Montpellier, CNRS, INSERM, Montpellier, France ; 2LabEx 'Ion Channel Science and Therapeutics', France ; 3Université Paris Cité, INSERM UMR1163, Imagine Institute, Paris, France ; 4INCI, Université de Strasbourg, CNRS, 67084 Strasbourg, France. Cav3.1 channels are low threshold activated T-type calcium channels. Several mutations in CACNA1G, the gene coding for Cav3.1 channels, have been identified in patients with SCA42ND (SpinoCerebellar Ataxia type-42 with Neurodevelopmental Deficits). This disease is associated with a marked atrophy of the cerebellum, as well as early severe motor and cognitive deficits. We have developed a conditional knock-in mouse model carrying the most recurrent SCA42ND mutation, p.Arg961Thr. The goal of the project is to characterize the phenotypical and histological properties of 2 mouse lines carrying the mutation, either globally or restricted to Purkinje cells (PC) of the cerebellum. We found that both lines exhibit a motor phenotype (using rotarod and beam tests), which was more severe in mice expressing the mutation ubiquitously. This study also points to the important role of Cav3.1 expressed in PCs in the SCA42ND motor phenotype. However, contrary to that reported in humans, no atrophy of the cerebellum was observed in the mice of the 2 lines, neither evidence for PC alteration. Further studies are ongoing to identify how the neuronal network in the cerebellum could be altered in this SCA42ND mouse model.

## P2 - STRUCTURAL INSIGHTS INTO GABAA RECEPTOR POTENTIATION BY QUAALUDE

**Weronika Chojnacka[1]**, Jinfeng Teng[2], Jeong Joo Kim[3], Jensen Anders[4], Ryan Hibbs[2]
*[1] Guillaume Lebon Lab, Institute of Functional Genomics, Montpellier, France; [2] Ryan Hibbs Lab, University of California San Diego, San Diego, USA; [3] Loxo@Lilly, Louisville, USA; [4] Anders Jensen Lab, University of Copenhagen, Copenhagen, Denmark*

Methaqualone, a quinazolinone marketed commercially as Quaalude, is a central nervous system depressant that was used clinically as an anxiolytic and sedative, then became a notorious recreational drug in the 1960s-80s. Due to its high abuse potential, methaqualone was outlawed for medical use, but remains a drug of abuse around the world. Methaqualone principally acts upon GABAA receptors, which are the major ionotropic inhibitory neurotransmitter receptors in the central nervous system. Its restricted status and limited accessibility have contributed to methaqualone's pharmacology being understudied. Here we use cryo-EM to localize GABAA receptor binding sites of methaqualone and its more potent derivative, PPTQ, to the same intersubunit transmembrane sites shared by the general anesthetics: propofol and etomidate. We observe these ligands inserting more deeply into subunit interfaces than previously-characterized modulators. We elucidate the mechanism of action of quinazolinones; their binding causes widening of the extracellular half of the ion-conducting pore, following a trend among positive allosteric modulators in destabilizing the hydrophobic activation gate in the pore as a mechanism for potentiation.

## P3 - UNVEILING SUBREGIONAL FEATURES OF THE SLOW AMPAR CURRENT IN THE HIPPOCAMPUS

**Marin Boutonnet[1]**, Niccolo Pampaloni[2], Andrew Plested J. R.[1]
*[1] Institüt für Biologie, Zellulare Biophysik, Humboldt Universität zu Berlin, Germany; [2] Charité Universitätsmedizin, Berlin, Germany*

Glutamatergic synapses play a fundamental role in hippocampus excitatory neurotransmission, with the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptors (AMPARs) driving fast synaptic responses. Notwithstanding extensive investigations into the function of AMPAR in synaptic transmission and plasticity, its pharmacology and opening kinetics in its native environment are not always clear. Indeed, interaction proteins fine-tune the spatial and temporal regulation of the AMPAR trafficking, current kinetics and function. A substantial proportion of AMPAR complexes contain auxiliary subunits, including TARPs or cornichons. For instance, the AMPAR co-expression with TARPs (such as TARPγ-2 or TARPγ-8) in heterologous system induces a superactivation of the receptor. Recently, our team revealed a surprisingly slow component of the AMPAR current driving a massive depolarization in mice hippocampal neurons and human cortex, opening a decisive new dimension for understanding excitatory network physiology. The expression profiles of AMPAR and auxiliary subunits vary considerably in the brain, resulting in a mosaic of possible protein complexes. Synaptic gene expression of the component of the AMPAR inner core varies remarkably in the hippocampus. Accordingly, we observed pedestal currents in ⅓ of CA1 pyramidal neurons, but never in dentate gyrus granule cells suggesting a regulated AMPAR complex expression. Nonetheless, the composition of AMPAR protein complexes underpinning the slow AMPAR current remains unknown. In the present work, we characterized further the slow AMPAR current in the hippocampus. By using one-photon glutamate uncaging on organotypic hippocampal slice cultures, we reported the slow AMPAR current is preferentially expressed in pyramidal neurons of CA1/3 of the ventral hippocampus. Following the hypothesis that this gradient of expression is associated with differential transcriptomic identities, we have analysed publicly-available RNAseq data with respect to AMPA receptor subunits and their interactome. Combined with a functional analysis of pyramidal cell responses, these results give new insight into AMPA receptor diversity and its effect on synaptic transmission in the hippocampus Overall, this work aims shedding light on neuronal heterogeneity and synapse diversity in the hippocampus. The understanding of these powerful synapses provides new perspectives on the computational power of hippocampal networks, and in the related cognitive processes, including memory.

### P4 - SELECTIVE PHOTOMODULATION OF GLUN1/GLUN2A/GLUN2B TRI-HETEROMERS USING PHOTOSWITCHABLE COMPOUNDS

**Sophie Shi,** Laetitia Mony, Pierre Paoletti
*Paoletti team, Institut de Biologie de l'Ecole Normale Supérieure (IBENS), École Normale Supérieure, Université PSL, CNRS-INSERM, France*

Ionotropic glutamate receptors (iGluRs) are key mediators of excitatory neurotransmission in the mammalian brain. Among them, NMDA receptors (NMDARs) are calcium-permeable channels whose activation requires both the binding of two agonists—glutamate and glycine—and membrane depolarization to relieve the magnesium block. NMDARs play essential roles in brain development, synaptic plasticity, and cognitive function, and their dysfunction has been implicated in several neuropsychiatric disorders, including schizophrenia and epilepsy. NMDARs are heterotetramers composed of two obligatory GluN1 subunits and two GluN2 subunits (GluN2A, 2B, or 2C), with each GluN2 subtype conferring distinct biophysical, pharmacological, anatomical, and signaling properties. Discriminating between the roles of di-heteromeric NMDARs (e.g., GluN1/GluN2A/GluN2A or GluN1/GluN2B/GluN2B) and tri-heteromeric NMDARs (e.g., GluN1/GluN2A/GluN2B) is crucial to better understand receptor function in specific brain regions and their involvement in neuropsychiatric conditions. In particular, GluN1/GluN2A, GluN1/GluN2B di-heteromers, and GluN1/GluN2A/GluN2B tri-heteromers represent the three major NMDAR subtypes expressed in the mouse hippocampus. Several studies highlight the importance of the GluN2A-to-GluN2B subunit ratio as a key regulator of neural processes, including bidirectional synaptic plasticity, and susceptibility to excitotoxic damage. However, the respective contributions of GluN2A and GluN2B di-heteromers versus GluN2A/GluN2B tri-heteromers in these mechanisms remain poorly understood and a subject of ongoing debate.  In our lab, we aim to selectively target GluN2A/2B tri-heteromeric NMDARs using an optogenetic pharmacology approach. This strategy combines two key techniques: thiol-maleimide conjugation for covalent drug attachment and azobenzene-based photoswitchable drug for light-controlled receptor modulation. Together, these methods result in a designed drug compound offering strong molecular selectivity, high spatiotemporal resolution and cell-type specificity. Some initial screening results of photoswitchable compounds using two-electrode voltage clamp in Xenopus laevis oocytes expressing GluN1/GluN2A, GluN1/GluN2B di-heteromers, and GluN1/GluN2A/GluN2B tri-heteromers are obtained. Two types of photoswitchable compounds were tested: one based on a 2B-selective positive allosteric modulator, spermine, and another based on a bifunctional maleimide joined in the middle by an azobenzene linker. Several hits were obtained, exhibiting a low photomodulation ratio, with selectivity for the GluN1/GluN2A/GluN2B tri-heteromers.

### P5 - TRAAK CHANNEL: UNLOCKING THEIR ROLE IN NOCICEPTION

**Kelly Jeanne,** Franck Chatelain, Florian LESAGE, Delphine BICHET
*Institut of cellular and molecular pharmacology (IPMC), Valbonne, France*

Chronic Pain is a major issue in our society as it affects 20 million peoples by years in France and significantly impacts their quality of life. It causes physical and psychological suffering, limits daily activities, and leads to social isolation. It also represents a significant economic burden by increasing healthcare costs. Moreover, pain management raises ethical challenges, particularly with opioid use, highlighting the need for effective and accessible alternative treatments. It's crucial to investigate ion channels involved in modulating pain whose expression is highly specific and limited to structures involved in nociception. This is the case for TRAAK (Twik-Related Arachidonic Acid-activated K+ channel). This member of two-pore domain family, play a crucial role in neuronal function and nociceptive signal regulation. TRAAK is particularly interesting due to its mechano- and thermosensitive properties and its expression in sensory neurons, making these channels promising therapeutic targets. Our project focuses on the implication of TRAAK (Twik-Related Arachidonic Acid-activated K+ channel) in nociception and investigates whether specifically targeting this channel, rather than other K2P channels, could provide new pain management strategies. In this first year of Ph.D., we demonstrate that TRAAK channels is expressed in nociceptive fiber and colocalize with nociceptors. We also examine the impact of TRAAK inactivation (KO TRAAK) and its over-activation through a gain-of-function (TRAAK KI) mutation in mice. If we demonstrate that TRAAK over-activation in primary sensory fibers reduces nociceptive sensitivity, this could open avenues for developing pharmacological TRAAK activators for pain treatment.

## P6 - DE NOVO VARIANTS OF NALCN DIFFERENTIALLY IMPACT BOTH THE PHENOTYPIC SPECTRUM OF PATIENTS AND THE BIOPHYSICAL PROPERTIES OF THE NALCN CURRENT

Nawale Hadouiri[1], Lidiane Pereira Garcia[2], Romain Baudat[1], Philippe Lory[1], Christelle Thauvin-Robinet[3], **Arnaud Monteil[1]**

*[1] Department of Physical Medicine and Rehabilitation, Dijon-Bourgogne University Hospital, Dijon, France; [2] Institute of Functional Genomics, University of Montpellier, CNRS, INSERM, Montpellier, France; [3] UMR-Inserm 1231, Génétique des Anomalies du Développement (GAD), Bourgogne Franche-Comté University, Dijon, France*

The Na+ leak channel NALCN regulates the resting membrane potential and consequently cell excitability of several cell types, including neurons. Studies of animal models demonstrated that NALCN is involved in fundamental physiological functions such as respiratory rhythm, circadian rhythm, sleep, locomotor behavior and pain perception. Pathogenic variants of NALCN have been associated with ultra-rare developmental disorders characterized by a wide range of symptoms with variable severity. We and others previously showed that pathogenic variants of NALCN can be categorized in 2 groups. The first group corresponds to inherited biallelic loss-of-function variants with patients suffering from the IHPRF1 syndrome (OMIM #615419). The second one corresponds to de novo gain-of-function variants that cause the CLIFAHDD syndrome (OMIM #616266). In this study, we provide a standardized phenotypic description of a large group of 35 individuals with de novo pathogenic variants of NALCN. In addition, we performed functional studies of several of these variants using the patch clamp technique in a recombinant system. We highlight a large heterogeneity in terms of both expressed symptoms and their severity. By contrast with previous reports only showing a pure gain-of-function effect of de novo pathogenic variants, we found that de novo variants of NALCN differentially impact the biophysical properties of the NALCN current and likely influence cell excitability. To conclude, de novo variants of NALCN differentially impact the biophysical properties of the NALCN current. We hypothesize that this may at least partly explain the phenotypic diversity observed in patients.

**P7 - TWO PORE DOMAIN THIK2 CHANNEL IS INVOLVED IN ACUTE AND CHRONIC PAIN REGULATION**

Nicolas Gilbert, Franck Chatelain, Solène Gibaud, Thomas Lorivel, Marie-Emmanuelle Kerros, Sylvain Feliciangeli, Florian Lesage, **Delphine Bichet**
*Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne, France*

Two-pore domain potassium channels (K2P) are crucial regulators of neuronal excitability. Among them, THIK1 and THIK2 remain poorly characterized. Unlike other K2P channels, their role in pain modulation is still unclear, though their specific expression in nociceptive neurons and microglia suggests a potential involvement in pain pathways. This reserach project explores the functional role of THIK channels in pain pathophysiology using molecular, electrophysiological, and behavioral approaches. First, we mapped THIK1/2 expression in the mouse peripheral nervous system and analyzed sensory neuron properties in THIK2 knock-out (KO) mice. Results showed that THIK2 deficiency increases neuronal excitability, particularly under inflammatory conditions. Behavioral pain tests in THIK2-KO mice revealed heightened thermal hypersensitivity, supporting a protective role of THIK2 in inflammation-induced pain. Similar tests in THIK1-KO mice indicated acute inflammatory hypersensitivity. Microglial analysis showed reduced reactivity in THIK1-KO mice and heightened activation in THIK2-KO mice under inflammation. Overall, our findings identify THIK2 as a key modulator of inflammatory pain and a promising peripheral therapeutic target for chronic pain treatment without central opioid-related side effects.

**P8 - COLD-ACTIVATED SPINAL CIRCUITRY AND TRP CHANNEL CONTRIBUTION IN CHEMOTHERAPY INDUCED PERIPHERAL NEUROPATHY (CIPN)**

**Marina Villaro**, Félix Viana, Ana Gomis
*Instituto de Neurociencias, UMH-CSIC, Sant Joan d'Alacant, Spain*

Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent and disabling side effect of neurotoxic anticancer agents, characterized by peripheral nerve damage. Among its painful symptoms, cold hypersensitivity is particularly severe, often leading to dose reduction or treatment discontinuation, with serious implications for patient survival and well-being. While peripheral mechanisms—such as ion channel dysregulation in primary afferent neurons—have been extensively studied, much less is known about how these changes affect central processing at the spinal cord level. This project investigates cold-evoked neuronal activation patterns in the dorsal horn, using a mouse model of CIPN induced by oxaliplatin. Neuronal activation was permanently tagged in TRAP2-iCreERT2-tdTomato mice during exposure to innocuous and noxious cold stimuli. A second stimulation under identical conditions was performed to determine the temporal stability of cold-induced neuronal activation, assessed by cFos staining. Additionally, to investigate the contribution of cold-sensitive ion channels, naïve mice received intraplantar injections of AITC (a TRPA1 agonist) or WS12 (a TRPM8 agonist), followed by analysis of spinal neurons activated by noxious cold. Behavioral assays confirmed the development of cold hypersensitivity in CIPN mice. At the spinal level, oxaliplatin-treated animals showed increased numbers of tdTomato+ neurons following cold stimulation, with a higher degree of co-localization with cFos after a second noxious cold exposure. Interestingly, cFos labelling alone did not differ significantly between conditions, suggesting possible limitations in sensitivity or temporal dynamics between markers.

Pharmacological activation of TRPA1 and TRPM8 nerve endings in naïve mice revealed that both agonists recruit comparable numbers of dorsal horn neurons, with partial overlap with the neuronal populations activated by noxious cold in both cases. These results suggest that both channels contribute to spinal cold processing but do not fully define the cold-responsive neuronal ensemble. Overall, these findings reveal stat-dependent changes and cold-evoked spinal circuit activation during CIPN and highlight the value of TRAP2-based activity tagging for studying persistent circuit alterations. Further studies, integrating molecular profiling and electrophysiological approaches, will be essential to identify specific neuronal subpopulations involved in cold hypersensitivity.

Keywords: oxaliplatin, dorsal horn, cold hypersensitivity, TRPA1, TRPM8, neuron labelling.

## P9 - DEVELOPMENT OF A CELLULAR MODEL FOR PHARMACOLOGICAL SCREENING OF COMPOUNDS TARGETING THE NALCN CHANNELOSOME

**Nattaya Thongsepee**, Romain Baudat, Philippe Lory, Arnaud Monteil
*Institute of Functional Genomics, Montpellier, France*

The sodium leak channel (NALCN) is a critical regulator of resting membrane potential and cellular excitability in neurons and endocrine cells. Gain-of-function de novo pathogenic variants in NALCN are linked to severe neurodevelopmental disorders characterized by a broad spectrum of symptoms. Partial inhibition of NALCN has been proposed as a potential therapeutic strategy; however, no selective pharmacological modulators have been identified to date. This lack of progress is largely due to the absence of a robust expression system compatible with high-throughput drug screening, as NALCN requires at least three auxiliary subunits for functionality and its constitutive expression is toxic to HEK293 cells. To address this challenge, we developed a multitransposon-based platform to generate HEK293 cell lines—both wild-type and mutant—that stably express the complete NALCN channelosome under inducible control. Notably, we discovered that NALCN current expression is cell cycle-dependent, enabling the optimization of conditions for consistent and reliable electrophysiological recordings. Using these novel cell lines, we have initiated the characterization of pharmacological properties of the NALCN channelosome, with several candidate molecules currently under extensive evaluation. Our work provides a foundational tool for the discovery of NALCN-targeting compounds and opens new avenues for therapeutic development—not only for NALCN-associated neurodevelopmental disorders, but potentially also for conditions such as psychiatric disorders, chronic pain, and cancer.

## P10 - SLOW DYNAMICS FROM FAST ACTIVATING KINETICS IN PACEMAKING NEURONS

Arthur Fyon[1], Olesksandra Pavlova[2], Nick Schaar[3], Alessio Franci[1], Pietro Mesirca[4], Matteo Mangoni[4], Jochen Roeper[3], Vincent Seutin[2], Guillaume Drion[1], **Kevin Jehasse[1]**
*[1] Laboratory of Neuroengineering, University of Liège, Belgium; [2] Laboratory of Neurophysiology, GIGA-Neurosciences, University of Liège, Belgium; [3] Institute of Neurophysiology, Neurosciences Center, Goethe University of Frankfurt am Main, Germany; [4] Institut de Génomique Fonctionnelle, Université de Montpellier, France;*

Pacemaking is the ability of excitable cells to fire action potentials spontaneously without any external stimulation. Pacemaking cells can be divided in two groups: fast pacemakers and slow pacemakers. While fast pacemaking is well understood, the mechanism of slow pacemaking remains controversial. To sustain such slow frequency of firing, a small amplitude current is needed during the interspike interval. Recent work showed that the pacemaker current does not result from cooperative channels, but from a newly identified small conductance pore sensitive to the gating-pore blocker 1-(2,4-xylyl)guanidinium (XG) in midbrain dopamine neurons. We therefore investigated in the mechanism is similar across species and in sinus node cells. We recorded pacemaking activity in human dopamine neurons derived from induced-pluripotent stem cells and mouse sinus node cells and observed that pacemaking is silenced by XG. Then, we used a conductance-based model of dopamine neurons to study the dynamics of the pacemaking conductance (gPace). We adjusted activation kinetics of sodium and calcium conductances to physiological values and observed that, without gpace, the model could not generate spontaneous activity nor slow frequency firing. Moreover, gPace restored slow pacemaking over a wider parameter space, even when conventional mechanisms failed. Our simulations revealed that only transient sodium, delayed rectifier potassium channels and gPace are required to produce slow pacemaking and, interestingly, that only rapid activation kinetics of gPace can support slow frequency firing. These observations were then confirmed using dynamic-clamp. These findings suggest that gPace is crucial for sustaining slow firing, supporting a shared mechanism for slow pacemaking driven by rapid activation of gPace.

### P11 - THE ROLE OF NAV1.8 IN HYPERHIDROSIS

**Jolien Vander Cruyssen[1],** Suguru Yamauchi[2], Michele Cervellera[3], Ruslan I. Dmitriev[3], David Valle[4], Malcolm V. Brock[2], Frank Bosmans[5]
*[1] Molecular Physiology and Neurophysics group, Department of Basic and Applied Medical Sciences, Faculty of Medicine and Health Sciences, University of Ghent, Ghent 9000, Belgium; [2] Department of Surgery, School of Medicine, Johns Hopkins University, Baltimore, MD, USA; [3] Tissue Engineering and Biomaterials Group, Department of Human Structure and Repair, Faculty of Medical and Health Sciences, Ghent University, C. Heymanslaan 10, 9000, Ghent, Belgium; [4] McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD, USA; [5] Experimental Pharmacology group (EFAR), Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, Vrije Universiteit Brussel, Brussels 1050, Belgium*

Primary Idiopathic Hyperhidrosis (PIH) is a common but poorly understood disorder marked by excessive diurnal sweating. Despite affecting an estimated 2–5% of the population, its underlying mechanisms have remained elusive, hindering development of effective therapies. We identified a genetic and mechanistic basis for PIH, demonstrating that electrical remodeling and hyperactivation of the sympathetic nervous system (SyNS) underlies disease pathogenesis. Whole-exome sequencing of PIH patients revealed an enrichment of variants in voltage-gated sodium (NaV) channels, with NaV1.8 (SCN10A) emerging as a key candidate. Focusing on a clinically penetrant NaV1.8 variant, we generated a transgenic mouse model that phenocopies the excessive sweating observed in human PIH patients. Molecular mapping confirmed NaV1.8 expression in thoracic sympathetic ganglia in both humans and mice, and electrophysiological analyses showed that the NaV1.8 mutation enhanced channel function. Calcium imaging of dissociated thoracic sympathetic ganglia revealed exaggerated cholinergic responses consistent with sympathetic hyperexcitability. Importantly, the excessive sweating phenotype was fully reversed by non-selective NaV channel inhibitors as well as a NaV1.8-specific research compound. These findings establish PIH as an ion channelopathy characterized by sympathetic overactivity, with NaV1.8 representing a targetable node within a broader excitability pathway that may be influenced by additional ion channels or regulatory proteins.

### P12 - MAKING AN AMPA RECEPTOR SLOW

**Jennifer D. Noonan,** Irene Riva, Andrew J. R. Plested
*Institut für Biologie, Humboldt Universität zu Berlin, Germany*

AMPA receptors are known for their fast kinetics. But recently AMPA receptors displaying slow currents that decay in 500 ms and are resistant to desensitization have been reported in brain slices. At the synapse, receptors are in complex with auxiliary proteins that modulate receptor kinetics and likely underpin the slow AMPA response. However, it is not known what specific subunits or auxiliary proteins contribute, in particular because no known combination of subunits produces such slow kinetics (about 500x slower than the usual decay). By utilizing a chimeric TARP of gamma-2 and gamma-8 (termed super TARP) that exerts super-active effects on the AMPA receptor, we observed a subunit-dependent modulation of GluA2(R):GluA1 heteromers. The superTARP specifically slowed kinetics of GluA2(R) homomeric receptors, but not GluA1. In the GluA2(R): GluA1 heteromer, the superTARP again slowed receptors kinetics, confirming that not only do TARPs exhibit subunit-dependent modulation of receptor function, but also indicating a dominant role for GluA2 in the heteromer. The super TARP also introduced polyamine block in heteromeric receptors. However, the responses generated were not slow enough to account for slow AMPA currents in neurons. In an effort to identify additional subunit combinations that produce slow AMPA responses, we turned to published RNAseq data from the hippocampus and tested subunits with heterogeneous expression patterns in hippocampal pyramidal neurons. Surprisingly, the "fast" AMPA subunit GluA4, presumed absent from pyramidal neurons, in combination with various auxiliary proteins showed the slowest decays measured to date. This work highlights new types of AMPA receptor diversity and identifies novel potential mediators of the slow AMPA response.

### P13 - STRUCTURAL INSIGHTS INTO THE DIVERSE ACTIONS OF MAGNESIUM ON NMDA RECEPTORS

Xuejing Huang[1]; **Xiaole Sun[1]**; Qinrui Wang[2]; Jilin Zhang[1]; Han Wen[2]; Wan-jin Chen[3]; Shujia Zhu[4]
*[1] Institute of Neuroscience, Shanghai, China; [2] DP Technology, Beijing, China; [3] Department of Neurology and Institute of Neurology of First Affiliated Hospital, Fuzhou, China; [4] Southern University of Science and Technology, Shenzhen, China*

Magnesium ($Mg^{2+}$) is a key regulatory ion of N-methyl-D-aspartate (NMDA) receptors, including conferring them to function as coincidence detectors for excitatory synaptic transmission. However, the structural basis underlying the $Mg^{2+}$ action on NMDA receptors remains unclear. Here, we report the cryo-EM structures of GluN1-N2B receptors and identify three distinct $Mg^{2+}$-binding pockets. Specifically, site Ⅰ is located at the selectivity filter where an asparagine ring forms coordination bonds with $Mg^{2+}$ and is responsible for the voltage-dependent block. Sites Ⅱ and Ⅲ are located at the N-terminal domain (NTD) of the GluN2B

subunit and involved in the allosteric potentiation and inhibition, respectively. Site Ⅱ consists of three acidic residues, and the combination of three mutations abolishes the GluN2B-specific $Mg^{2+}$ potentiation, while site Ⅲ overlaps with the $Zn^{2+}$ pocket, and mutations here significantly reduce the inhibition. Our study enhances the understanding of multifaceted roles of $Mg^{2+}$ in NMDA receptors and synaptic plasticity.

## P14 - DVL2 INFLUENCES AXON INITIAL SEGMENT FORMATION, ORGANIZATION AND ACTION POTENTIAL GENERATION

Sonia Poirrault-Chassac[1], Juliette Villagomez[1], Sophie Belmonte[1], Ana Dorrego-Rivas[1], Nathalie Aubailly[1], Charlotte Isch[1], Nathalie Sans[1], Mireille Montcouqiol[1], **Arne Battefeld[2]**, Jerome Ezan[1]

*[1] Univ. Bordeaux, INSERM, Magendie, U1215, F-33000 Bordeaux, France; [2] Univ. Bordeaux, CNRS, IMN, UMR 5293, F-33000 Bordeaux, France*

The planar cell polarity (PCP) signaling pathway organizes apical-basal polarity in epithelial cells. In the central nervours system, disruptions of this pathway have been found to lead to neural tube closure defects or epilepsy. In previous work, we have identified Pk2, a member of the core PCP pathway, as a modulator of axon initial segment (AIS) formation and neuronal excitability. However, the molecular role and function of other PCP proteins in neurons remains obscure, but putatively include cellular organization through cytoskeleton reorganization. The AIS is a crucial neuronal subdomain important for establishing the axo-dendritic polarity and as initiation site for action potentials (AP). This is achieved by clustering high densities of voltage gated sodium and potassium channels via the highly specific underlying cytoskeleton. We here performed a screen for other members of the PCP family and identified that the core PCP protein Dishevelled2 (Dvl2) accumulates at the AIS in vitro and in vivo. We observed Dvl2 co-localization with microtubules (MT), implying a potential impact in the fasciculation of microtubules (MT). The downregulation of Dvl in vitro impaired AIS formation and maintenance by altering the distribution and levels of AnkG followed by a rearrangement of AIS Nav channels. This cyto-morphological change was functionally associated with a strong reduction of the AP initiated at the AIS and changes of AP properties. AP changes were maintained during prolonged knock-down. Taken together, Dvl2 is important for establishing and maintaining AP generation at the AIS, by mediating interactions between the cytoskeleton and voltage gated ion channels clustered at the AIS.

## P15 - THE DEUBIQUITINATING ENZYME UCH-L1 REGULATES NEURONAL CA2+ SIGNALLING

**Marie Perrier[1],** Desirée Loreth[2], Johannes Brand[2], Laurence Aubry[1] , Edward W.Tate[3,4], Gian-Marvin Kipka[5,6], Malte Gersch[5,6], Catherine Meyer-Schwesinger[2], Marie-Odile Fauvarque[1], Alexandre Bouron[1]

*[1] Université Grenoble Alpes, CNRS, CEA, Inserm, IRIG, BGE UA13, F-38000 Grenoble, France; [2] Institute for Cellular and Integrative Physiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [3] The Francis Crick Institute, London, UK; [4] Imperial College London, Department of Chemistry, Molecular Sciences Research Hub, London, UK; [5] Chemical Genomics Centre, Max-Planck-Institute of Molecular Physiology, Dortmund, Germany; [6] Dept. of Chemistry and Chemical Biology, TU Dortmund University, Dortmund, Germany*

Ubiquitination influences a myriad of biological processes like the trafficking or degradation of ubiquitin-tagged target proteins. This post-translational modification can be reversed by deubiquitinating enzymes (DUBs) that counterbalance the action of E3 ubiquitin ligases. We investigated the impact of PR-619, a membrane-permeable and broad-spectrum DUBs inhibitor, on the entry of Ca2+ through native voltage-gated Ca2+ channels (VGCCs) of cultured embryonic cortical neurons. Fura-2-based Ca2+ imaging experiments showed that PR-619 reduced the cytosolic Ca2+ rises induced by depolarization by affecting mainly dihydropyridine-sensitive (L-type) VGCCs. This inhibition was sensitive to the dynamin inhibitor Myr-Dip and the lysosomal agent chloroquine and bafilomycin-A. Hence, PR-619 reduced the amount of Cav1.2 proteins. A pharmacological approach was set out to better delineate the identity of the DUB responsible of this inhibitory PR-619 action. Since UCH-L1 and USP19 are two highly expressed neuronal DUBs, we investigated the effects of selective UCH-L1 (IMP1710, GK13S) and USP19 (ADC141) inhibitors. IMP1710 and GK13S depressed the Ca2+ uptake through L-type VGCCs whereas ADC141 and the UCH-L3 inhibitor TCID had no effects. In addition, UCH-L1 inhibition impaired the neuronal Ca2+ storage capacities of neurons. Thus, UCH-L1 influences the neuronal uptake and storage of Ca2+, which is likely to have important physiological implications. Altogether, these results posit UCH-L1, the main DUB of the brain, as an important regulator of the neuronal Ca2+ homeostasis and add to our understanding of its cellular functions.

## P16 - ROLE OF VOLTAGE-SENSING DOMAIN IV IN β1-MEDIATED MODULATION OF NAV1.6 GATING

**Maxime Lammens[1]**, Frank Bosmans[1], Filip Van Petegem[2]
*[1] Molecular Physiology and Neurophysics Group, Department of Basic and Applied Medical Sciences, Ghent University, Ghent, Belgium; [2] Department of Biochemistry and Molecular Biology, the Life Sciences Centre, University of British Columbia, Vancouver, BC, Canada*

The voltage-gated sodium (Nav) channel subunit 1.6 associates with multiple proteins, including β-subunits (SCN1B-SCN4B), which are capable of modulating channel function and pharmacology. Among these auxiliary subunits, β1 transiently interacts with the channel thereby affecting its trafficking to the plasma membrane and modifying channel gating. Reflecting its biological importance, mutations in SCN1B have been associated with disorders such as cardiac defects and epilepsy syndromes. Nav1.6 consist of four homologous regions, each containing a voltage-sensing domain (VSD) and a pore-forming domain (PD). Structural data indicates binding of β1 to VSDIII within Nav1.6, yet how this interaction alters channel gating remains poorly understood. Moreover, there is increasing evidence that VSDIV is also involved in β1 modulation of Nav channels. However, the underlying molecular determinants have yet to be identified. To address this, we expressed Nav1.6 VSDIV mutants and a chimeric construct with β1 in Xenopus laevis oocytes and assessed subunit-induced modulation using the two-electrode voltage-clamp method. A single charge-reversal mutation of a conserved extracellular glutamate residue (E to K) in VSDIV of Nav1.6 almost completely abolished β1 modulation of the conductance-voltage (G-V) relationship and significantly reduced its effect on channel availability. Interestingly, channel inactivation kinetics and recovery from fast inactivation remained unaffected indicating that partial β1 interaction may still occur. Charge neutralization produced similar effects on Nav1.6 gating, thereby highlighting the importance of this conserved glutamate. However, inserting the glutamate-containing VSDIVS3-S4 loop of

Nav1.6 into Nav1.8 did not enhance its susceptibility to β1 modulation. These findings underscore the functional significance of a conserved extracellular glutamate residue and suggest a previously underappreciated role for VSDIV in β1 modulation of Nav channel gating.

## P17 - PIEZO ACTIVATION LEADS TO CARDIAC ARRHYTHMIA

**Victor Bernard**
*Institut de Génomique Fonctionnelle - CNRS UMR 5203, Montpellier, France*

Mechanical forces are linked to heart development and function such as cardiac rhythmicity, necessary to ensure proper blood flow. Recent studies have demonstrated that PIEZO1, a mechanosensitive ion channel protein, plays a role in calcium homeostasis and signalling in mouse cardiomyocytes. Furthermore, overexpression of Piezo1 has been linked to severe heart failure and ventricular tachycardia, suggesting a potential connection between PIEZO1 and cardiac arrhythmias. To explore whether activating Piezo1 could cause disruptions in cardiac rhythm, we employed a combination of pharmacological approaches and various cardiac analyses in zebrafish. Our findings reveal that prolonged activation of Piezo1 by YODA agonist leads to notable cardiac arrhythmias. In human, activating mutations of PIEZO1 have been associated with xerocytosis, a hemolytic anemia, due to erythrocyte dehydration. Interestingly, we identified a PIEZO1 variant in a Turkish patient suffering from both xerocytosis and defect in heart rhythmicity. We aim to characterize this variant and study its potential involvement in cardiac arrhythmia.

## P18 - IN VIVO FUNCTIONAL AND TRAFFICKING ANALYSIS OF HERG MISSENSE VARIANTS IN A CRISPR/CAS9-ENGINEERED C. ELEGANS MODEL OF CONGENITAL LONG-QT SYNDROME

Antoine Delinière[1], Clément Boiteux[2], Sara Hysa[3], Sara Sechi[3], Elie Nevoret[3], Philippe Chevalier[2], Thomas Boulin[3], **Olga Andrini[3]**
*[1] Centre de référence national des troubles du rythme cardiaque héréditaires ou rares de Lyon (CERA), Institut de Cardiologie des Hospices Civils de Lyon, Hôpital Louis Pradel, Bron, France; [2] Service de Rythmologie, Institut de Cardiologie des Hospices Civils de Lyon, Hôpital Louis Pradel, Bron, France; [3] Université Claude Bernard Lyon 1, CNRS UMR 5284, INSERM U1314, MeLiS, Lyon, France*

Loss-of-function variants in KCNH2, which encodes the hERG K+ channel, underlie long QT syndrome type 2 (LQT2), a leading cause of sudden death in young individuals. Yet, 78% of hERG missense variants remained of uncertain significance, underscoring the need for scalable assays. UNC-103, the C. elegans ortholog of hERG, exhibits high conservation in sequence, structure, and key regulators of channel biogenesis. To quantify the trafficking efficiency and functional impact of hERG missense variants in vivo, we developed a CRISPR/Cas9-engineered C. elegans model. Using fluorescent UNC-103 knock-in strains, we established an approach combining quantitative confocal imaging with behavioural assays of muscle hyperexcitability. We analysed well known pathogenic variants located in the pore domain of hERG and characterised a novel variant recently published by our team (Delinière A. et al., Gene, 2024), uncovering distinct mechanism-specific profiles. This physiologically relevant approach bridges the gap between in vitro assays and complex transgenic animals, enabling in vivo variant characterisation and opening new avenues for therapeutic screening in LQTS.

## P19 - DISCOVERY OF HNAV1.2 MODULATORS THROUGH LARGE-SCALE SPIDER VENOM SCREENING

Floriane Bibault[1], Jérôme Montnach[1], Lucie Jaquillard[2], Massimo Mantegazza[3-5], Rémy Béroud[2], Barbara Ribeiro De Oliveira[1]; Michel De Waard[1]
*[1] Institut du Thorax, Inserm UMR 1087 / CNRS UMR 6291, Nantes, France; [2] Smartox Biotechnology, Saint-Egrève, France; [3] Université Côte d'Azur, Valbonne-Sophia Antipolis, France; [4] CNRS UMR7275, Institute of Molecular and Cellular Pharmacology (IPMC), Valbonne-Sophia Antipolis, France; [5] Inserm U1323, Valbonne-Sophia Antipolis, France*

Voltage-gated sodium channels are essential for the transmission of nerve impulses. Among them, hNaV1.2, encoded by the SCN2A gene, plays a critical role in the propagation of action potentials in the developing brain. Mutations in SCN2A often result in a loss of channel function or exert a dominant-negative effect, whereby the mutated channel interferes with the function of the wild-type channel, further impairing neuronal activity. These disruptions are strongly associated with severe neurodevelopmental disorders, including autism spectrum disorders and schizophrenia. Currently, no targeted therapies exist for hNaV1.2 dysfunction. Given that ion channels are common targets of natural venoms, we implemented a screening strategy using a large venom library to identify specific modulators of the hNaV1.2 channel. Our goal was to discover natural molecules capable of restoring or enhancing the function of hNaV1.2 channels impaired by pathogenic mutations. Eighteen spider venoms from diverse geographical origins were fractionated into 64 fractions each using reversed-phase HPLC. From each venom, the 46 most hydrophobic fractions were selected, yielding a total of 828 primary fractions. These were individually screened using high-throughput patch-clamp electrophysiology on HEK cells stably expressing human hNaV1.2 (n=8 per fraction). Automated analysis of activation and inactivation protocols, performed in R language, identified 53 active fractions: 24 activators and 26 inhibitors. These active fractions were found to modulate the biophysical properties of the channel, including current amplitude, activation, and inactivation - mechanisms consistent with known venom interactions on sodium channels. We then selected 47 of the most promising fractions, re-fractionated them using cation-exchange HPLC, and conducted a second screening round. The final set of active fractions was submitted for sequencing to identify the peptides of interest. This study represents the first large-scale screening of venom-derived modulators specifically targeting human NaV1.2. The identified peptides will next be evaluated for selectivity across other Nav isoforms. Ultimately, we aim to identify novel Nav1.2-selective peptides with therapeutic potential for the treatment of brain disorders.

## P20 - MODELING PHOTOPHARMACOLOGICAL CONTROL OF HERG FUNCTION IN THE HUMAN HEART

**Elouan Voisin**, Matthias Dereli, Navneet Roshan, Jérôme Montnach, Michel De Waard, Rupamanjari Majumder
*Institut du Thorax, Nantes, France*

The hERG potassium channel plays a critical role in cardiac repolarization and is a key determinant of the QT interval. Genetic mutations in hERG can cause hERG channel dysfunction, leading to long-QT syndrome—a disorder that increases the risk of dangerous arrhythmias. While light-based therapies are advancing rapidly in neuroscience, their

potential in heart rhythm modulation remains largely untapped. In this study, we explore a novel photopharmacological strategy by modeling how BeKm-1, a light-activated peptide, interacts with hERG channels in heart tissue. We develop a computational framework that simulates both the light-controlled binding of BeKm-1 and its impact on electrical conduction in the human heart. Using this model, we show how spatially targeted light exposure can disrupt normal rhythm and trigger a severe arrhythmia known as Torsades de Pointes. Our results highlight the potential—and risks—of using light to precisely control heart activity, opening new avenues for non-invasive cardiac therapies that integrate molecular pharmacology with optical control.

## P21 - SCREENING AND OPTIMIZATION OF CELL-PENETRATING PEPTIDES FOR DELIVERY IN HUMAN CARDIOMYOCYTES

**Serena Hachem**, Flavien Charpentier, Michel De Waard
*L'Institut du thorax, Université de Nantes, France*

Cardiac arrhythmias are often linked to abnormal expression or function of voltage-gated sodium channels, particularly Nav1.5, encoded by the SCN5A gene, a key determinant of action potential initiation and propagation in cardiomyocytes. Mutations and dysregulations of SCN5A gene often lead to severe cardiac arrhythmias, including Brugada syndrome. However, many therapeutic candidates face critical delivery challenges, particularly in cardiac cells. In this context, Cell-Penetrating Peptides (CPPs) have emerged as a powerful class of short peptides capable of transporting various cargo molecules across cellular membranes, including proteins, nucleic acids, and small drugs. Their favorable physico-chemical properties and non-viral nature make them attractive candidates for therapeutic delivery, especially in hard to transfect cells such as cardiomyocytes. Despite their potential, CPP efficiency varies widely depending on sequence, structure, and cellular context, underscoring the need for careful screening and optimization. This project aims to evaluate and to assess the uptake, localization and distribution of a panel of CPPs in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), a physiologically relevant in vitro model. For this, ten fluorescently labeled CPPs were tested across 3 different cell lines: AC16 (immortalized human ventricular cardiomyocytes), isolated neonatal mouse cardiomyocytes, and hiPSC-CMs. Cellular uptake and subcellular distribution were first evaluated qualitatively using fluorescence microscopy. To assess uptake efficiency more quantitatively, fluorescence signal was measured using a plate reader, on both AC16 and hiPSC-CMs cells. To date, we have demonstrated that several of the tested CPPs can successfully enter cardiomyocytes via different mechanisms. Our preliminary data enabled us to identify a subset of peptides with favorable uptake profiles. This analysiscontributes to improving the delivery efficiency of CPP, and allows us to select efficient CPPs as promising candidates and for subsequent conjugation to cargo molecule of choice. Looking forward, the selected CPP will serve as a delivery vector for promising tools for gene regulation. Among the considered molecules, Peptide Nucleic Acids (PNAs) emerged as new potential transcriptional modulators of the SCN5A gene. Our study supports and lays the foundation for future gene therapies strategies based on CPP-mediated delivery in cardiac cells.

Keywords: Cell-Penetrating Peptides (CPP), Screening, Intracellular Delivery, Cardiac Cells, Peptide uptake

**P22 - ORGANOTYPIC CARDIAC SLICES FROM HUMAN HEARTS AS A PLATFORM FOR ANTIARRHYTHMIC DRUG TESTING**

**Anthony Cote**, Jayce Phillips, Peter Kohl, Ursula Ravens, Remi Peyronnet
*Institute of Experimental Cardiovascular Medicine, University Medical Center Freiburg, Germany*

Introduction: Organotypic cardiac slices provide a promising approach for short- and long-term drug screening. Our aim is to validate this model by testing the electrophysiological effects of standard antiarrhythmic drugs: flecainide (class 1c antiarrhythmic) and dofetilide (class 3 antiarrhythmic) in slices of human hearts. Methods: Left ventricular tissue excised from patients was sliced into 400 µm thick sections, mounted in biomimetic chambers, and paced electrically at a frequency of 1 Hz. A preload of 1 mN was applied. Action potentials (AP) were recorded at 37°C using intracellular microelectrodes pulled from borosilicate glass capillaries (tip resistance 15-20 MΩ when filled with 3 M KCl solution). The bath solution contained (in mM): NaCl 140, KCl 5.4, HEPES 10, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 10. Observations before, during and after drug interventions are all paired. All values are reported as mean ± standard deviation. Results: AP from slices had resting membrane potentials (RMP) of -83.7 ± 5.5 mV, AP amplitudes (APA) of 110.4 ± 7.1 mV, maximal depolarization speeds (dV/dtmax) of 104.3 ± 45.7 mV/ms, AP durations at 30% of repolarization (APD30) of 214.6 ± 24.0 ms, APD50 of 327.2 ± 58.1 ms and APD90 of 460.1 ± 72.4 ms (N=8 hearts, n=18 slices). The triangulation index was measured at 1.17±0.26. Thus, AP exhibited consistent shapes during phase 3 though the APD50 and APD90 values varied considerably among patients. Flecainide caused a reversible decrease in dV/dtmax of 25.8 ± 13.2% at 3 µM (N=3, n=6) while APD90 was not significantly altered. Dofetilide reversibly increased APD90 by 22.2 ± 3.2% at 10 nM, and by 50.1 ± 3.9% at 30 nM (N=1, n=2). Conclusion: The organotypic cardiac slice model produced consistent results with regard to RMP, APA values, and AP shape showed clear triangularisation. Although other AP parameters, such as dV/dtmax and individual APD varied among patients, known antiarrhythmic drugs tested produced the expected effects. The results obtained in this study suggest that the slice model is suitable for testing of drug effects on key parameters of cardiac electrophysiology, making this model promising for assessing novel antiarrhythmic drugs.

**P23 - DEVELOPMENT AND VALIDATION OF HUMAN FRAGMENT ANTIBODIES TO THE NEONATAL VARIANT OF THE CARDIAC SODIUM CHANNEL (NNAV1.5)**

**Samir Hamaia[1]**, William Brackenbury[2], Christopher Huang[3], **Antony Jackson[1], Samantha Salvage[1,4]**
*[1] Department of Biochemistry, University of Cambridge, CB2 1QW, UK; [2] York Biomedical Research Institute, Department of Biology, University of York, York, YO10 5DD, UK; [3] Department of Physiology, Development and Neuroscience, University of Cambridge, CB2 3EL, UK; [4] St George's School of Health and Medical Sciences, City St George's, SW17 0RE, UK*

Emerging evidence indicates expression of the neonatal variant of the cardiac voltage-gated sodium channel (nNav1.5) in many cancers, particularly those of the colon and breast. It appears to be an early event in cancer evolution, occurring upstream of a network of invasion associated genes and sodium-dependent metabolic changes, contributing to metastatic potential [1 - 4]. Due to a mutually exclusive alternative splicing event, nNav1.5 differs by just

7 amino acids to the adult isoform (Nav1.5), mainly within the S3-4 extracellular loop of the Domain I voltage-sensing module. Beyond early developmental stages, nNav1.5 is not expressed in healthy tissues, except minimally in breast tissue [3].
Thus, antibodies that uniquely identify nNav1.5 would be very useful reagents for detection and therapy.
Both polyclonal and monoclonal antibodies have been generated against nNav1.5, and utilised in foundational research [3, 5-6], however there are none commercially available. Using a phage-display human single chain variable fragment (scFv) library (>1013 independent clones) we have screened and selected for nNav1.5 specific scFvs using peptide sequences across the variant extracellular loop. Our first ELISA screen has identified 16 scFvs which selectively bind the neonatal peptide, but not the adult peptide. These scFvs have been sequenced and 12 unique clones will be taken forward for further screening against the native protein transiently expressed in HEK293 cells.
Those scFv(s) which display suitable affinity and selectivity can be used for both mechanistic research purposes or taken forward for development as early diagnostic tools.

1. House CD, Vaske CJ, Schwartz AM, Obias V, Frank B, Luu T, et al. Voltage-gated Na+ channel SCN5A is a key regulator of a gene transcriptional network that controls colon cancer invasion. Cancer Res. 2010;70(17).
2. Lastraioli E, Fraser SP, Guzel RM, Iorio J, Bencini L, Scarpi E, et al. Neonatal nav1.5 protein expression in human colorectal cancer: Immunohistochemical characterization and clinical evaluation. Cancers (Basel). 2021;13(15).
3. Yamaci RF, Fraser SP, Battaloglu E, Kaya H, Erguler K, Foster CS, et al. Neonatal Nav1.5 protein expression in normal adult human tissues and breast cancer. Pathol Res Pract. 2017;213(8).
4. Leslie TK, Tripp A, James AD, Fraser SP, Nelson M, Sajjaboontawee N, et al. A novel Nav1.5-dependent feedback mechanism driving glycolytic acidification in breast cancer metastasis. Oncogene. 2024 Aug;43(34):2578–94.
5. Brackenbury WJ, Chioni AM, Diss JKJ, Djamgoz MBA. The neonatal splice variant of Nav1.5 potentiates in vitro invasive behaviour of MDA-MB-231 human breast cancer cells. Breast Cancer Res Treat [Internet]. 2007 Jan 12 [cited 2017 Feb 16];101(2):149–60. Available from: http://link.springer.com/10.1007/s10549-006-9281-1
6. Chioni AM, Fraser SP, Pani F, Foran P, Wilkin GP, Diss JKJ, et al. A novel polyclonal antibody specific for the Nav1.5 voltage-gated Na+ channel "neonatal" splice form. J Neurosci Methods. 2005;147(2).

## P24 - MODULATION OF C6 GLIOMA CELL MOTILITY BY THE VOLTAGE-GATED SODIUM CHANNEL β3 SUBUNIT

Hengrui Liu, Samir Hamaia, Lisa Dobson, Federico López Hernández, Christopher Beaudoin, Samantha Salvage, Christopher l-H Huang, Laura Machesky, **Antony Jackson**
*Department of Biochemistry, University of Cambridge, CB2 1QW, UK*

Voltage-gated sodium channels (VGSCs) initiate action potentials in nerve and muscle cells and are regulated by regulatory β subunits. VGSC β subunits are also expressed in some cancer types, suggesting potential functions distinct from their role in electrophysiological excitability. This study investigated the occurrence and functional implications of the VGSC β3

subunit (the product of SCN3B gene) in glioma. Data mining revealed negative correlations between SCN3B gene expression and glioma severity and aggressiveness. Expression of β3 in the C6 glioma cell line reduced cell migration and invasion without affecting cell viability. Filopodia were significantly increased while lamellipodia/ruffles were decreased, producing striking cell morphological changes. These effects were abrogated by expression of the β3 subunit lacking its Ig domain and by the exogenous application of a single-chain variable fragment (scFv) antibody targeting the β3 Ig domain. Most of the plasma membrane-associated proteins immunoprecipitated with the β3 subunit and identified by mass spectrometry are known regulators of actin polymerization.
Our data reveal a novel and unexpected role for the VGSC β3 subunit in orchestrating actin organization and negatively regulating cell migration in glioma cells that may potentially explain clinical correlations with glioma severity.

### P25 - BASAL O-GLCNACYLATION IS ESSENTIAL TO ENSURE PROPER ELECTRICAL ACTIVITY AND CALCIUM HANDLING IN RAT VENTRICULAR CARDIOMYOCYTES

Matthieu Douard[1], Emma Abell[1], Floriane Bibault[1], Sabine Charron[1], Pierre Dos Santos[1], Ed White[2], Fanny Vaillant[1], **Fabien Brette[3]**
*[1] IHU Liryc, Pessac, France; [2] University of Leeds, UK; [3] INSERM U1046 PhyMedExpAim, Montpellier, France*

O-GlcNAcylation, a post-translational modification of proteins, has emerged as an important modulator of physiological functions. In the heart, there is evidence that O-GlcNAcylation is involved in cardiac diseases. Surprisingly, little is known on O-GlcNAcylation and its regulation by hexosamine biosynthetic pathway (HBP) in healthy cardiac myocyte, hence it's implication for excitation-contraction coupling. Here we address the role of O-GlcNAcylation and the enzymes in this pathway (O-GlcNAc transferase/O-GlcNAcase) on cardiac activity.
Methods & Results: We used physiological (glucosamine) and pharmacological approaches (O-GlcNAc transferase/ O-GlcNAcase inhibitors OSMI-1 and Thiamet-G respectively) to modulate HBP. Cardiac ventricular myocytes were enzymatically isolated. Electrical activity was measured using the patch clamp technique (action potential (AP)) and calcium handling was assessed by calcium transients (CaTr) and sparks recordings. Western blotting was used to quantify O-GlcNAcylation levels.
The perfused, whole, working heart configuration was used to assess cardiac function. Short-term exposure (10 min) with glucosamine and Thiamet-G had no effect on O-GlcNAcylation levels, AP and CaTr duration. However, long-term exposure (4 hours) to glucosamine and Thiamet-G increased O-GlcNAcylation levels, decreased AP and CaTr duration. In contrast, short- and long-term exposure with OSMI-1 decrease O-GlcNAcylation levels and induced a large AP and CaTr duration prolongation. These effects were accompanied by spontaneous AP, higher calcium spark and wave frequencies. Hearts perfused with glucosamine and Thiamet-G had similar electrical and hemodynamic properties as control while OSMI-1 induced QT prolongation, then failing heart.
Conclusions : A basal level of O-GlcNAcylation is crucial for proper electrical and calcium handling properties in the heart.

**P26 - GENETIC ABLATION OF L-TYPE CAV1.3 CHANNELS IN CARDIAC CONDUCTION SYSTEM INDUCES BRADYCARDIA AND INCREASED SUSCEPTIBILITY TO ATRIAL FIBRILLATION.**

**Marvin Gaillardon**, Mélanie Faure, Audrey Dubourg, Isabelle Bidaud, Kai Schönig, Dunan Bartsch, Matteo Mangoni
*Institut de Génomique Fonctionnelle, Montpellier, France*

The spontaneous activity of pacemaker cells in sinoatrial node (SAN) controls the heart rhythm under physiological conditions. Several studies by our group show the relevance of the L-type Ca2+ channel Cav1.3 on the generation of pacemaker activity and in electrical conduction system within the heart. Atrial fibrillation (AF) is the most frequent cardiac arrythmia, often associated with an electrical remodeling. So far, the underlying electrophysiological mechanism is not fully understood.
Here we aim to evaluate the impact of global genetic Cav1.3 deletion or its selective deletion in the conduction system, on the AF susceptibility.
We generated mice carrying conditional floxed Cav1.3 allele (Cav1.3 Flex eGFP / Flex eGFP) and crossed with HCN4 CreERT2 line allowing deletion of Cav1.3 in cardiac HCN4 expressing cells (HCN4+) upon tamoxifen injection (Cav1.3 eGFP/eGFP mice) within conduction system. The second mice model is a Cav1.3 KO allowing a complete deletion of Cav1.3 in all cardiac tissue. We investigated the effect of Cav1.3 deletion on heart automaticity by telemetric ECGs and Langendorff-perfused hearts.
Then, susceptibility to AF was study by using a Langendorff-perfused heart coupled to optical mapping. S1-S9 train pulses stimulation protocols were used to trigger AF. Cav1.3 eGFP/eGFP mice displayed in vivo significant bradycardia (464± 35 BPM in untreated group vs 377±31BPM in tamoxifen group) as well as ex vivo (411±50 BPM vs. 278±78 BPM), associated with an increase of AF susceptibility (22% in untreated group vs 56% in tamoxifen group) compared to untreated group.
Similarly, the global deletion of Cav1.3 in the heart lead to slower automaticity (348±74 BPM in WT mice vs. 224±55 BPM in Cav1.3 KO) and increase arrythmias such as atrio-ventricular (AV) blocks and AF susceptibility. In sum, our data show that deletion of Cav1.3 leads to impaired electrical conduction and heart rate generation. Loss of Cav1.3 causes major rhythms disorders such as bradycardia increased heart rate variability, AV blocks, and seems to increase significantly AF susceptibility.
This study underscores the major role of Cav1.3 in cardiac conduction system. Thus, Cav1.3 channel could be a relevant target to improve AF treatment.

**P27 - MODELLING SUPRAMOLECULAR CLUSTERS OF VOLTAGE-GATED SODIUM CHANNELS IN THE CARDIOMYOCYTE PERINEXAL SPACE**

**Christopher Beaudoin**, Christopher Huang, Antony Jackson
*Department of Biochemistry, University of Cambridge, UK*

The members of the voltage-gated sodium channel family (Nav1.1-1.9) are known to carry action potentials along nerve and muscle fibers. A Nav channel comprises a single chain of four internally homologous domains that enclose a central pore, which determines ion selectivity and passage dynamics. Additionally, each channel domain contains a voltage sensing domain (VSD) that allosterically regulates the tertiary structure – resulting in the opening and closing of the selectivity pore – in response to changes in membrane voltage.

Four accessory β-subunits (β1-4) have been discovered to interact with Nav channels to modulate voltage gating, kinetics, trafficking, and localization.
The β-subunit structures are characterized by an N-terminal extracellular Ig-like domain, a transmembrane domain, and a C-terminal intracellular tail – all of which been shown to interact with Nav channels.
Previous findings by the Jackson and other groups have revealed that the Ig-like domains of the β-subunits can oligomerize while the transmembrane domains may, nevertheless, bind to the Nav channels. Furthermore, our recently published data suggest that N-linked glycans on Nav1.5, which is primarily expressed in cardiomyocytes, may prevent the β1 and β3 Ig-like domains from interacting with the channel and induce β-subunit oligomerization while the transmembrane domains may still interact. Therefore, the binding of β-subunit oligomers to Nav1.5 may form networks of channels that permit specialized and localized action potential generation in cardiomyocyte membranes – particularly in the perinexal space.
Using protein structure and plasma membrane modelling and molecular dynamics simulations, the interactions between Nav channels/β-subunit clusters on the same and opposing membranes were investigated to shed light on the structural and electrophysiological effects of such multimerization.
These modelling efforts provide novel insights into the perinexal space and its effect on action potential conduction.

## P28 - FUNCTIONAL CHARACTERIZATION OF LYSOSOMAL ION CHANNELS AND TRANSPORTERS USING AUTOMATED PATCH CLAMP AND SOLID-SUPPORTED MEMBRANE ELECTROPHYSIOLOGY

**Artem Kondratskyi**
*Nanion Technologies, Munich, Germany*

Lysosomal ion channels and transporters are critical regulators of pH, membrane potential, calcium signaling, and autophagy, playing central roles in maintaining cellular homeostasis. Their dysfunction is implicated in numerous diseases, including lysosomal storage disorders, neurodegeneration, cancer, and infections. As such, they are emerging as important therapeutic targets, driving the need for scalable, high-throughput methods for functional analysis. Recent advances in automated patch clamp (APC) and solid-supported membrane electrophysiology (SSME) have made it possible to study the functional properties of lysosomal channels and transporters with greater precision and throughput. In this context, channels such as TRPML1, TMEM175, and TPC2, as well as transporters like Cystinosin and SLC15A4, have come under increasing investigation.
Here, we present novel functional data on Cystinosin, TRPML1, and TPC2, obtained using both APC and SSME platforms. These findings highlight the power of combining complementary electrophysiological techniques to advance our understanding of lysosomal transport proteins and accelerate drug discovery targeting lysosomal dysfunction.

**P29 - DO CLC-2 CHLORIDE CHANNELS PLAY A ROLE IN HUMAN PANCREATIC DUCTAL ADENOCARCINOMA?**

Concetta Altamura[1], Ilaria Saltarella[1], Paola Laghetti[1], Simone Dell'Atti[1], Giulia Maria Camerino[2], Maria Raffaella Greco[1], Maria Antonia Frassanito[3], Rosa Angela Cardone[4], **Jean-François Desaphy[1]**
*[1] Section of Pharmacology, Department of Precision and Regenerative Medicine, University of Bari Aldo Moro, Bari, Italy; [2] Section of Pharmacology, Department of Pharmacy – Drug Sciences, University of Bari Aldo Moro, Bari, Italy; [3] Section of Clinical Pathology, Department of Precision and Regenerative Medicine, University of Bari Aldo Moro, Bari, Italy; [4] Department of Biosciences, Biotechnologies, and Biopharmaceutics, University of Bari Aldo Moro, Bari, Italy*

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers. Despite recent progress of combined chemotherapy, new therapies are urgently needed. Ion channels have been pinpointed as potential regulators of tumor cell proliferation, invasiveness, and chemotherapy resistance. We investigated the role of ClC chloride channels in human pancreatic adenocarcinoma cell lines (Panc-1, Mia-PACA), cancer stem cells (CSC) derived from Panc-1, and healthy pancreatic cell line (HPDE). Using RT-PCR, we found a greater transcript expression of ClC-2 and ClC-7 chloride channels and their respective auxiliary subunits HEPACAM and Ostm1 in tumoral cell lines compared to HPDE. Overexpression of ClC-2 protein was confirmed by WB in cancer cell lines. Using patch-clamp, whole-cell chloride currents resembling ClC-2 were recorded in ~25 % of patched Panc-1 cells and CSC (n=55 each), whereas no chloride current was found in HPDE cells (n=22). These currents were blocked by meclofenamic acid (MCFA), a well-known ClC-2 inhibitor (inhibition >50 % at 30 µM). Using flow cytofluorometry, we observed that 3 days incubation of CSC with MCFA reduced cell proliferation and increased cell apoptosis. These effects were low but significant with 30 µM MCFA and very marked at higher MCFA concentrations (100-300 µM). Running Kaplan-Meier plotter on 150 PDAC patients, we observed that high levels of ClC-2 expression were associated with a shorter Disease-Free Survival. These findings strongly suggest ClC-2 channel as a negative prognostic marker in PDAC, identifying it as a potential therapeutic target for this devastating disease. Supported by Italian Ministry of University (PRIN n. 20174TB8KW-002).

**P30 - CALCIUM CHANNELS IN GLIOBLASTOMA STEM CELLS**

Théo Branchu[1], Laetitia Cousin[1], Thomas Harnois[1], Antony Cunha[2], Sébastien Papot[2], Bruno Constantin[1], Valérie Coronas[1], **Nadine Déliot[1]**
*[1] 4CS Laboratory UR 22751, Université de Poitiers, France; [2] Institut de Chimie des Milieux et Matériaux de Poitiers, France*

Glioblastoma (GBM) is the most aggressive and common malignant brain tumor in adults, characterized by rapid growth and resistance to conventional therapies. Treatment typically involves radiotherapy, chemotherapy with temozolomide (TMZ), and surgery; however, median survival remains poor at approximately 15 months. Tumor recurrence occurs in about 90% of patients. This relapse has been attributed to the presence of cancer stem cells, a subpopulation responsible for tumor initiation, progression, and treatment resistance. Intracellular calcium signaling plays a pivotal role in regulating key cellular processes such as proliferation, migration, and self-renewal in glioblastoma stem cells (GSCs).

Our previous studies demonstrated the involvement of store-operated calcium entry (SOCE) in the physiology of GSCs, making it an attractive therapeutic target. Consequently, the pharmacological inhibition of SOCE by carboxyamidotriazole (CAI) has been investigated in GSCs. CAI is an oral inhibitor of non-voltage-dependent calcium channels and has been evaluated in a multicenter phase IB clinical trial in combination with TMZ. In human GSCs, CAI inhibits SOCE in a dose-dependent manner and regulates proliferation, self-renewal, and migration. To specifically target brain tumors, a prodrug delivery strategy has been developed by the chemistry team led by S. Papot in Poitiers. The cellular mechanism of the prodrug's activity has been studied in our model to develop an in vivo tool for targeting GSCs and delivering an inhibitor of SOC/Orai. Other calcium entry pathways include receptor-operated channels (ROCs) and stretch-activated channels (SACs). ROCs, particularly TRPC3 and TRPC6, are functional in GSCs and may also contribute to the regulation of GSC properties. Among SACs, Piezo1, TRPV4, and TRPC5 are expressed in human GSCs and can be activated in pharmacological calcium imaging experiments. Altogether, our studies highlight the roles of SOC, ROC, and SAC in calcium signaling in human GSCs, suggesting that pharmacological inhibition of these channels could represent a promising strategy to target the GSC population responsible for the high relapse rate in glioblastoma

## P31 - TARGETING TRPV6/CXCR4 COMPLEXES PREVENTS CASTRATION-RESISTANT PROSTATE CANCER METASTASIS TO THE BONE

**Clément Cordier[1],** Aurélien Haustrate[1], Adriana Mihalache[2], Erika Duval[2], Emilie Desruelles[1], Corentin Spriet[3], Lotfi Slimani[4], Pierre Gosset[2], Natalia Prevarskaya[1], V'yacheslav Lehen'kyi[1]
*[1] Université de Lille, Inserm, U1003 - PHYCEL - Physiologie Cellulaire, F-59000, Lille, France, [2] Pathology department, Saint Vincent de Paul Hospital, Lille Catholic Hospitals, Lille, France; [3] University of Lille, CNRS, Inserm, CHU Lille, Institut Pasteur of Lille, US 41 - UAR 2014 - PLBS, F-59000, Lille, France; [4] University of Paris Cité and Sorbonne Paris Nord, Inserm UMR1333, Oral Health, F-92120 Montrouge, France*

Bone metastasis most commonly occurs in castration-resistant prostate cancer (CRPC). TRPV6 calcium channel is absent in healthy prostate tissue while its expression increases considerably during cancer progression. We hypothesized that cancer cell induces TRPV6 expression de novo to directly benefit from the tightly-regulated calcium intake via TRPV6 while providing cancer cell with a selective advantage for its metastasis in the calcium-abundant niche as bone. Using a cohort of prostate cancer tissues biopsies with the clinical history of patients for at least 10 years after the biopsy, we report that TRPV6 expression directly correlates with CRPC tumor aggressiveness and increased risk of metastasis development. TRPV6 channel was involved in the acquisition of both mesenchymal and invasive phenotypes through the increased phosphorylation of CaMK2 followed by the translocation of the transcription factor NF-κB to the nucleus and expression of EMT markers, MMPs, and transcriptional factors as Twist, Snail, and Slug. Moreover, TRPV6 expression was accompanied with the increased formation of CXCR4/TRPV6 complexes. In vivo, mice bearing trpv6+/+ tumors showed an increase in the metastasis occurrence, notably bone metastasis, whereas trpv6-/- developed no metastasis at all. Targeting TRPV6 using monoclonal antibody resulted in a significant reduction in metastasis burden and an increase in overall survival. Combined with the selective inhibitor of CXCR4 receptor, AMD3100, the synergistic effect in suppression of metastasis development was achieved. Thus, the suppression of CRPC metastasis

dissemination to the bone can be achieved via simultaneous targeting of TRPV6/CXCR4 demonstrating a combined therapy proof-of-concept in vivo.

### P32 - CHALLENGING A DOGMA: USELESSNESS OF VOLTAGE-GATED NA+ CHANNELS FOR FAST MUSCLE FIBERS OF ADULT ZEBRAFISH TO TWITCH

Léa Demesmay[1], Romane Idoux[1], Christine Berthier[1], Claire Bernat[2], Léon Espinosa[3], Vincent Jacquemond[1], Frédéric Brunet[4], Philippe Lory[2], Bruno Allard[1], **Sophie Nicole[2]**

*[1] Physiopathologie et Génétique du Neurone et du Muscle, CNRS 5261, INSERM U1315, Université Lyon 1, Lyon, France; [2] Institut de Génomique Fonctionnelle, Université de Montpellier, CNRS 5203, INSERM U1191, Montpellier, France; [3] Laboratoire de chimie bactérienne, CNRS 7283, Aix-Marseille Université, Marseille, France; [4] Institut de Génomique Fonctionnelle de Lyon, ENS Lyon, CNRS 5242, INRA USC1370, Université Lyon 1, Lyon, France*

Fast skeletal muscle fibers from zebrafish share a number of functional properties with mammalian twitch muscle fibers, making this vertebrate a precious model to investigate the pathophysiology of neuromuscular disorders. In this study, we explored the properties of voltage-gated Na+ channels (NaV) responsible for initiation and propagation of AP in isolated adult zebrafish fast skeletal muscle fibers using voltage-clamp and current-clamp techniques and compared them to mouse fast twitch muscle fibers. We found that kinetics of activation and inactivation of NaV were faster in zebrafish fibers and, overall, that the voltage-dependence of inactivation was shifted by 25 mV toward negative voltages as compared to mouse fibers, yielding a mean half-inactivation potential of -90 mV. In agreement with these findings, recording of APs at various resting membrane potentials indicated that APs vanished for resting membrane potentials less negative than -80 mV in zebrafish, whereas APs could still be elicited from resting membrane potentials as low as -60 mV in mice. In addition, Ca2+ transients induced by field stimulation were insensitive to Na+ current blockade in zebrafish but not in mouse fibers. Fluorescence labelling of nicotinic acetylcholine receptors showed that zebrafish fast fibers were multi-innervated with mean distance between extra-synaptic sarcolemma and motor endplates of 14 µm, expected to lead to negligible attenuation of depolarization propagated from endplates. Finally, knock out of the two genes encoding pore-forming NaV subunits in zebrafish muscles did not induce any change in locomotion and escape behavior of the animals. Taken together, these data question the role of NaV and the occurrence of APs in zebrafish fast muscle.

### P33 - PRECLINICAL EVALUATION OF THE EFFECTS OF SAFINAMIDE ON THE ION CHANNEL FUNCTION IN MYOTONIC ADR MOUSE MODEL

Ileana Canfora[1], Letizia Claudione[1], Silvia Vailati[2], Carla Caccia[2], Elsa Melloni[2]; Gloria Padoani[2], Concetta Altamura[3], Jean-Francois Desaphy[3], **Sabata Pierno[1]**

*[1] Section of Pharmacology, Department of Pharmacy and Drug Sciences, University of Bari Aldo Moro, Bari, Italy; [2] Global Medical Office and R&D Department, Zambon S.p.A., Bresso, MI, Italy; [3] Section of Pharmacology, Department of Biomedical Sciences and Human Oncology, School of Medicine, University of Bari Aldo Moro, Bari, Italy*

Non-dystrophic myotonias are caused by mutations in the SCN4A and CLCN1 genes encoding the Nav1.4 sodium and ClC-1 chloride channels expressed in skeletal muscle. Myotonia is

characterized by muscle stiffness and pain. Mexiletine, a sodium channel blocker, is the drug of first choice in the treatment of myotonias. However, some patients have little benefit from mexiletine, for its side effects and contraindications. Safinamide has been identified as a potential antimyotonic drug. Safinamide (XADAGO®, ZAMBON) is indicated for the treatment of mil-to-late Parkinson's disease in combination with levodopa. However, this drug is also able to reduce the excessive release of glutamate through sodium channel blockade (Desaphy et al., Exp. Neurol, 328:113287, 2020). Safinamide effects were tested in an animal model of Myotonia Congenita, the "arrested development of righting response" (ADR) mouse. As in Myotonia Congenita, this model is characterized by genetically determined loss of function of the ClC-1 channel. Only the homozygous adr/adr mouse is symptomatic and shows myotonic signs, which can be appreciated by the difficulty to straighten themselves on four legs from the supine position. At the age of 4 weeks, homozygous mice were treated with safinamide to evaluate the Time of Righting Reflex (TRR). Safinamide was injected intraperitoneally at 10 mg /kg. The effects of safinamide were compared with those of saline solution and mexiletine 10 mg/kg (i.p.). The TRR was measured 20 min earlier and 20, 60, 90 and 120 minutes after drug injection. Safinamide exerted a more potent antimyotonic effect than mexiletine. The TRR value was significantly reduced by 56 ± 3 % after 60 minutes and by 35 ± 11% after 120 minutes with 10 mg/kg safinamide. For comparison, the same dose of mexiletine was less effective, being the TRR value reduced by 47 ± 4 % after 60 minutes and by 25 ± 4 % after 120 minutes. These results show that safinamide exerts a potent effect in counteracting the typical sign of myotonia in vivo (muscle stiffness assessed by TRR) and support its therapeutic use as an effective alternative to mexiletine in non-dystrophic myotonias.

### P34 - VACUOLAR MALATE AND FUMARATE TRANSPORT THROUGH ATALMTS

**Elsa Demes-Causse**, Roxane Doireau, Paloma Cubero-Font, Alexis De Angeli
*Institut des Sciences des Plantes de Montpellier, Montpellier, France*

Plants fix a proportion of the carbon into organic acids (i.e. malate and fumarate). These organic acids are then used in many metabolic pathways (i.e. TCA cycle, regulation of cytosolic pH, osmotic pressure) and have a role in stomata movements too. Therefore, the storage of organic acids has to be tightly regulated. In this study, we focus on one ion transporter family for which some members are able to transport organic acids. The Aluminum-activated malate transporters (ALMTs) family is involved in crucial physiological functions of land plants. Arabidopsis thaliana counts 14 members, some localized at the plasma membrane and others at the vacuolar membrane. Here we analyzed single or multiple knock out plants for the main vacuolar AtALMTs (AtALMT9, AtALMT5 and AtALMT4) which are known to transport malate and/or fumarate into the vacuole. Some of the multiple knock out plants show severe growth defects with early senescent leaves. Therefore, we wanted to investigate the transport of malate and fumarate in plants lacking one or more vacuolar AtALMTs. By using the electrophysiological patch clamp technique on Arabidopsis thaliana vacuoles from each mutant, we analyzed the transport capacity of malate and fumarate in each of the mutants. When all three of the vacuolar transporters are knocked out, the transport of fumarate is almost completely impaired. This study shows that the vacuolar AtALMT channels are crucial for the regulation of organic acid accumulation in the vacuole.

**P35 - FRAMESHIFT MUTATION-INDUCED ALTERNATIVE TRANSLATION AS A MECHANISM FOR CARDIAC ARRHYTHMIA INHERITANCE**

__**Arnaud Landra-Willm**[1]__, Stephanie Häfner[2], Marion Bied[1], Crystal Guillemin[3], Chris Jopling[3], Guillaume Sandoz[1]

*[1] Institut de Biologie Valrose, Université Côte d'Azur, Nice, France; [2] Rudolf Schönheimer Institute of Biochemistry of the Faculty of Medicine, Universität Leipzig, Germany; [3] Institut de Génomique Fonctionnelle, Université de Montpellier, France*

Mutations in the cardiac potassium channel gene KCNQ1 are a major cause of cardiac arrhythmias and sudden death. Frameshift mutations typically introduce premature stop codons, resulting in C-terminally truncated (KCNQ1ΔCt) proteins that lack channel function. In dominant cases, these truncated proteins can multimerize with and inhibit wild-type (WT) KCNQ1 channels. However, the dominant inheritance of certain KCNQ1 frameshift variants remains unexplained, particularly when the resulting proteins lack the domains required for multimerization and dominant-negative action. Here, we identify frameshift mutation-induced alternative translation initiation (fsATI) as a pathogenic mechanism underlying this paradox. We show that the KCNQ1 variants c.573_577del (R192fs) and c.533delinsGG (A178fs) both introduce an in-frame ATG at position +443, leading to an alternative initiation of translation and the production of an unexpected N-terminally truncated isoform (KCNQ1ΔNt) in addition to the expected KCNQ1ΔCt protein. While the KCNQ1ΔCt isoform is inert, the KCNQ1ΔNt isoform which retains the multimerization domain, co-assembles with WT KCNQ1, and exerts a potent dominant-negative effect on channel function. In zebrafish, cardiac expression of the KCNQ1ΔNt isoform induces bradycardia, phenocopying KCNQ1-loss-of-function. Our findings establish fsATI as a generalizable mechanism for dominant-negative effects in KCNQ1 frameshift mutations. This work expands our understanding of translation-based mechanisms in inherited channelopathies and has important implications for the interpretation of genetic variants and the development of targeted therapies.

**P36 - AI-DRIVEN PIPELINE FOR PREDICTING THE DYNAMIC GATING PATHWAYS OF VOLTAGE-GATED ION CHANNELS**

__Julia Kacher__, Mounir Tarek

*LPCT, Université de Lorraine, France*

While high-resolution structures from techniques like cryo-electron microscopy have become increasingly available, understanding the dynamic function of voltage-gated ion channels (VGICs) remains a fundamental challenge. The transient intermediate conformations that connect resting and activated states are critical for defining a channel's unique gating properties and its role in disease, but their fleeting nature makes them difficult to resolve experimentally. While molecular dynamics (MD) simulations can model these transitions, they are often limited by immense computational costs and the difficulty of overcoming energy barriers within feasible timescales. To overcome these limitations, we have developed a novel deep learning pipeline that integrates AI with physics-based insights to efficiently predict the intermediate states of VGICs. Our approach employs a 1D convolutional autoencoder that learns a low-dimensional, interpretable representation of a channel's conformational

landscape from structural data of two distinct operational states, such as those from short MD simulations. By navigating this learned representation, our model generates novel structures, effectively mapping the transition pathway between the initial states. A key innovation of our pipeline is the loss function tailored to voltage-gated ion channels. This function incorporates not only traditional geometric error but also physics-based energy constraints and novel terms related to the channel's gating charge. Recognizing gating charge as a superior collective variable for this protein superfamily ensures our predictions are not just structurally plausible but also biologically meaningful. We have successfully validated this pipeline on the Kv1.2 channel voltage sensor domain and extended its application to the whole tetrameric Kv7.1 channel, a critical player in cardiac function. Our research provides a powerful computational tool to generate testable hypotheses about VGIC function. The predicted dynamic pathways offer crucial insights into how patient-derived mutations, implicated in channelopathies like epilepsy and cardiac arrhythmias, can alter gating mechanisms.

# ATTENDEES: CONTACT INFO

**AKISHEVA Alina**
+33 761401848
alina.akisheva@etu.univ-nantes.fr
L'Institut du thorax
NANTES - FRANCE

**ANDRINI Olga**
+33 649392856
olga.andrini@univ-lyon1.fr
Université de Lyon
LYON - FRANCE

**BARA Anissa**
+45 20843990
aba@sophion.com
Industriparken 39
BALLERUP - DENMARK

**BATTEFELD Arne**
+33 533514835
arne.battefeld@u-bordeaux.fr
Institut des Maladies Neurodégénératives
BORDEAUX - FRANCE

**BEAUDOIN Christopher**
+44 7769072013
christopher.beaudoin@st-annes.ox.ac.uk
University of Oxford
OXFORD - UNITED KINGDOM

**BECK Heinz**
+49 15158233552
heinz.beck@ukbonn.de
University of Bonn
BONN - GERMANY

**BENOIT Evelyne**
+33 663751939
evelyne.benoit@cea.fr
CEA de Saclay - DMTS - SIMoS
GIF-SUR-YVETTE - FRANCE

**BERLIN Shai**
+972 778871123
shai.berlin@technion.ac.il
T3-Technion Technology Transfer
HAIFA - ISRAEL

**BERNARD Victor**
+33 786152733
victor.bernard@igf.cnrs.fr
141 rue de la Cardonille
MONTPELLIER - FRANCE

**BERROU Mikael**
+49 970449000
mberrou@wpi-europe.com
World Precision Instruments
FRIEDBERG - GERMANY

**BIBAULT Floriane**
+33 647730562
floriane.bibault@univ-nantes.fr
L'Institut du thorax
NANTES - FRANCE

**BICHET Delphine**
+33 631064925
bichet@ipmc.cnrs.fr
IPMC - Université Côte d'Azur
VALBONNE - FRANCE

**BOGESKI Ivan**
+49 5513965520
ivan.bogeski@med.uni-goettingen.de
University of Göttingen
GÖTTINGEN - GERMANY

**BOUTONNET Marin Alexi**
+49 33749452114
marin.alexi.boutonnet@hu-berlin.de
Humboldt-Universität zu Berlin
BERLIN - ALLEMAGNE

**BRETTE Fabien**
+33 627777242
fabien.brette@inserm.fr
IHU-Liryc/INSERM U1045
BORDEAUX - FRANCE

**BULTYNCK Geert**
+32 16330215
geert.bultynck@kuleuven.be
KU Leuven
LEUVEN - BELGIUM

**CHARNET Pierre**
+33 448792066
pierre.charnet@cnrs.fr
Institut des Biomolécules Max Mousseron
MONTPELLIER - FRANCE

**CHOJNACKA Weronika**
+33 638042581
weronika.chojnacka@igf.cnrs.fr
141 - rue de la Cardonille
MONTPELLIER - FRANCE

**CHRISTENSEN Rasmus**
+45 93947918
rasmus.christensen@saniona.com
Saniona AB
Copenhagen - Denmark

**CONSTANTIN Bruno**
+33 549453527
bruno.constantin@univ-poitiers.fr
UAR N° 2038
POITIERS - FRANCE

**CORDIER Clément**
+33 320336423
clement.cordier@inserm.fr
Université de Lille - Inserm UMR1003
VILLENEUVE D'ASCQ - France

**CORRATGE-FAILLIE Claire**
+33 689563562
claire.corratge-faillie@cnrs.fr
IPSIM
MONTPELLIER - FRANCE

**COTE Anthony**
+49 76127063944
anthony.cote@uniklinik-freiburg.de
University Medical Center Freiburg
FREIBURG - GERMANY

**DE ANGELI Alexis**
+33 499613177
alexis.deangeli@crns.fr
CNRS - IPSiM
MONTPELLIER - FRANCE

**DELIOT Nadine**
+33 549453829
nadine.deliot@univ-poitiers.fr
Université de Poitiers
POITIERS - FRANCE

**DEMES-CAUSSE Elsa**
+33 677344682
elsa.demes-causse@cnrs.fr
CNRS - IPSIM
MONTPELLIER - FRANCE

**DEMION Marie**
+33 660266532
marie.demion@umontpellier.fr
371 avenue du Doyen G. Giraud
MONTPELLIER - FRANCE

**FARFARIELLO Valerio**
+33 320336207
valerio.farfariello@univ-lille.fr
Bat SN3 - Avenue Paul Langevin
VILLENEUVE D'ASCQ - FRANCE

**GAILLARDON Marvin**
+33 611021718
marvin.gaillardon@igf.cnrs.fr
141 Rue de la Cardonille
MONTPELLIER - FRANCE

**GOROSTIZA LANGA Pau**
+34 934020208
pau@icrea.cat
Baldiri Reixac 10
BARCELONA - SPAIN

**HACHEM Serena**
+33 228080110
serena.hachem@etu.univ-nantes.fr
8 Quai Moncousu
NANTES - FRANCE

**HILAIRE Cécile**
+33 661121582
cecile.hilaire@umontpellier.fr
INM INSERM U1298
MONTPELLIER - FRANCE

**HUANG Christopher**
+44 7899828816
clh11@cam.ac.uk
30 Garden Walk
CAMBRIDGE - UNITED KINGDOM

**DESAPHY Jean-Francois**
+39 805478455
jeanfrancois.desaphy@uniba.it
Via Nicolò Giudice n.8
CELLAMARE - Italia

**FAUCHERRE Adèle**
+33 434359253
adele.faucherre@igf.cnrs.fr
141 rue de la Cardonille
MONTPELLIER - FRANCE

**GIRAULT Alban**
+33 322827641
alban.girault@u-picardie.fr
Université de Picardie Jules Verne
AMIENS - FRANCE

**GUERINEAU Nathalie**
+33 434359250
nathalie.guerineau@igf.cnrs.fr
CNRS-IGF-141 rue de la Cardonille
MONTPELLIER - FRANCE

**HAMAIA Samir**
+44 7765893163
swh23@cam.ac.uk
University of Cambridge
CAMBRIDGE - UNITED KINGDOM

**HOENDEROP Joost**
+31 634150741
joost.hoenderop@radboudumc.nl
Department of Medical BioSciences.
NIJMEGEN - THE NETHERLANDS

**INCI KAVUM Nurcan**
+32 471501168
ninci@uliege.be
Av. Henri Warnant 19
LIEGE - BELGIUM

**INQUIMBERT Perrine**
+33 634374080
inquimbert@unistra.fr
inci upr3212
STRASBOURG - FRANCE

**JACKSON Tony**
+44 1223765951
apj10@cam.ac.uk
University of Cambridge
CAMBRIDGE - UNITED KINGDOM

**JEANNE Kelly**
+33 662774132
jeanne@ipmc.cnrs.fr
853 chemin de notre dame
VALLAURIS - FRANCE

**JEHASSE Kevin**
+32 4 366 28 04
kevin.jehasse@uliege.be
Allée de la Découverte 10
LIEGE - BELGIUM

**KACHER Yulia**
+33 645878886
yulia.kacher@univ-lorraine.fr
CNRS UMR 7019 - LPCT
VANDOEUVRE-LÈS-NANCY - FRANCE

**KERROS Marie-Emmanuelle**
+33 493957783
kerros@ipmc.cnrs.fr
660 route des Lucioles - Sophia-Antipolis
VALBONNE - FRANCE

**KONDRATSKYI Artem**
+49 33762415503
artem.kondratskyi@nanion.de
Nanion Technologies GmbH.
MUNICH - GERMANY

**LABRO Alain**
+32 93320034
alain.labro@ugent.be
Corneel Heymanslaan 10
GHENT - BELGIUM

**LACOMBE Benoit**
+33 659673306
benoit.lacombe@cnrs.fr
place viala
MONTPELLIER - FRANCE

**LAMMENS Maxime**
+32 489417164
maxime.lammens@ugent.be
Corneel Heymanslaan 10
GHENT - BELGIUM

**LANDRA-WILLM Arnaud**
+33 618114333
arnaud.landra-willm@univ-cotedazur.fr
Parc Valrose
NICE - FRANCE

**LEGENDRE Claire**
+33 695865779
claire.legendre@univ-angers.fr
3 rue Roger Amsler
ANGERS - FRANCE

**LEGROS Christian**
+33 662700383
christian.legros@univ-angers.fr
Lab. MITOVASC
ANGERS - FRANCE

**LEMONNIER Loïc**
+33 320336207
loic.lemonnier@inserm.fr
INSERM UMR1003
VILLENEUVE D'ASCQ - FRANCE

**LEWIS Anthony**
+44 02392842664
anthony.lewis@port.ac.uk
University of Portsmouth
PORTSMOUTH - UNITED KINGDOM

**LI Athena**
+33 759588660
ali@ipmc.cnrs.fr
660 route des Lucioles - Sophia-Antipolis
VALBONE - FRANCE

**LISSE Frans**
+32 471735029
lisse.frans@ugent.be
University of Ghent
GHENT - BELGIUM

**LORY Philippe**
+33 672403936
philippe.lory@igf.cnrs.fr
141 rue de la Cardonille
MONTPELLIER - FRANCE

**LOURADOUR Julien**
+41 762059148
julien.louradour18@gmail.com
Bühlplatz 5
BERN - SWITZERLAND

**LOUSSOUARN Gildas**
+33 228080150
gildas.loussouarn@univ-nantes.fr
8 quai Moncousu
NANTES - FRANCE

**MAJUMDER Rupamanjari**
+33 751124509
rupamanjari.majumder@univ-nantes.fr
8 quai Moncousu 1
NANTES - FRANCE

**MALECOT Lionel**
+33 672364541
malecot.l@micromecanique.fr
101 RUE PEREIRE
SAINT-GERMAIN-EN-LAYE - FRANCE

**MASSON Bastien**
+33 658569084
bmasson@oriabs.com
6 rue Jean Calvin
PARIS - FRANCE

**MESIRCA Pietro**
+33 434359246
pietro.mesirca@igf.cnrs.fr
Université de Montpellier
MONTPELLIER - FRANCE

**MONTEIL Arnaud**
+33 434359250
arnaud.monteil@igf.cnrs.fr
141 rue de la Cardonille
MONTPELLIER - FRANCE

**MONY Laetitia**
+33 144323753
laetitia.mony@ens.fr
IBENS
PARIS - FRANCE

**NICOLE Sophie**
+33 434359250
sophie.nicole@igf.cnrs.fr
Institut Genomique fonctionnelle
MONTPELLIER - FRANCE

**NOONAN Jennifer**
+49 1748213065
jennifer.noonan@mail.mcgill.ca
Humboldt Universität zu Berlin
BERLIN - GERMANY

**PERRIER Marie**
+33 438782236
marie.perrier@cea.fr
CEA Grenoble - Laboratoire BGE
GRENOBLE - France

**PETRIZZELLI Théo**
+33 434359250
theo.petrizzelli@igf.cnrs.fr
IGF - CNRS
MONTPELLIER - FRANCE

**PEYRONNET Remi**
+49 76127063953
remi.peyronnet@uniklinik-freiburg.de
University Medical Center Freiburg
FREIBURG - GERMANY

**PIERNO Sabata**
+39 3498203220
sabata.pierno@uniba.it
Università di Bari
CELLAMARE - ITALIA

**PREVARSKAYA Natalia**
+33 320336423
natacha.prevarskaya@univ-lille.fr
Université de Lille
VILLENEUVE D'ASCQ - FRANCE

**PREVOST Marie**
+33 683027195
marie.prevost@pasteur.fr
Institut Pasteur
VANVES - FRANCE

**RAPETTI-MAUSS Raphaël**
+33 789597357
raphael.rapetti-mauss@univ-cotedazur.fr
Université Cote d'Azur
NICE - FRANCE

**RIBEIRO DE OLIVEIRA Barbara**
+33 620528030
barbara.ribeiro@univ-nantes.fr
L'Institut du thorax
NANTES - FRANCE

**RIO Aline**
+33 619126770
aline.rio@univ-nantes.fr
L'Institut du thorax
NANTES - FRANCE

**ROUGIER Jean-Sébastien**
+41 316845314
jean-sebastien.rougier@unibe.ch
Universität Bern
BERN - SWITZERLAND

**SALVAGE Samantha**
+44 7840637702
ss2148@cam.ac.uk
University of Cambridge
CAMBRIDGE - UNITED KINGDOM

**SCHROEDER Julian**
+1 8585347759
jischroeder@ucsd.edu
University of California San Diego
LA JOLLA - CA - USA

**SEUTIN Vincent**
+32 491113705
v.seutin@uliege.be
Université de Liège
LIEGE - BELGIUM

**SHI Sophie**
+33 621672015
sshi@bio.ens.psl.eu
IBENS
PARIS - FRANCE

**SIPIDO Karin**
+32 16330815
karin.sipido@kuleuven.be
KU Leuven
LEUVEN - BELGIUM

**SUN Xiaole**
+86 18993193557
sunxl2022@ion.ac.cn
Institute of Neuroscience
SHANGHAI - CHINA

**SZABO Ildiko**
+39 0498276324
ildiko.szabo@unipd.it
Università di Padova
PADOVA - ITALY

**TABAK Joel**
+44 1392723903
j.tabak@exeter.ac.uk
University of Exeter
EXETER - UNITED KINGDOM

**TAILLEBOIS Emiliane**
+33 668679983
emiliane.taillebois@univ-orleans.fr
Université d'Orléans
ORLÉANS - FRANCE

**THIREAU Jerôme**
+33 681506971
jerome.thireau@inserm.fr
Université de Montpellier - INSERM U1046
MONTPELLIER - FRANCE

**THONGSEPEE Nattaya**
+33 749547268
nattaya.thongsepee@igf.cnrs.fr
Institut de Génomique Fonctionnelle
MONTPELLIER - FRANCE

**TYPOU Alexandra**
+33 605558444
alexandra.typou@igf.cnrs.fr
Institut de Génomique Fonctionnelle
MONTPELLIER - FRANCE

**VANDENBERG Jamie**
+61 431895647
j.vandenberg@victorchang.edu.au
Victor Chang Cardiac Research Institute
WILLOUGHBY - AUSTRALIA

**VANDER CRUYSSEN Jolien**
+32 478752267
jolien.vandercruyssen@ugent.be
University of Ghent
ZWIJNAARDE - BELGIUM

**VILLARO Marina**
+34 685103847
mvillaro@umh.es
Instituto de Neurociencias, UMH-CSIC
SANT JOAN D'ALACANT - SPAIN

**VOISIN Elouan**
+33 780019499
elouan.voisin@univ-nantes.fr
Université de Nantes
NANTES - FRANCE

**WOOD John**
+44 7944621128
jnwoodfrs@gmail.com
University College London
LONDON - UNITED KINGDOM

**ZEEB Vadim**
+41 795260901
admin@nanthermix.com
NanThermix SA
LAUSANNE - SWITZERLAND

**ZHILINSKAYA Natalia**
+41 795260901
natalia@nanthermix.com
NanThermix SA
LAUSANNE - SWITZERLAND

**ZHU Shujia**
+86 13681605559
zhusj@sustech.edu.cn
Southern University of Sc. and Technology
SHENZEN - GUANGDONG - CHINA

# USEFUL INFORMATION

The attendees are expected on Sunday, between 4PM and 7PM. During this period, they would have the time to proceed to their check-in and meet each other before a welcome drink and the diner. The congress will begin on sunday evening with the plenary lecture and finish on Wednesday after lunch.

The conference will take place at “Centre de vacances du Lazaret”, a leisure center at Sète on the Mediterranean coast of France, close to the city of Montpellier.

### Directions to "Le Lazaret"

**Le Lazaret**
La Corniche
223 Rue Pasteur Benoît
34200 Sète

**Tel**: +33 (0)4 67 53 22 47
**Fax**: +33 (0)4 67 53 36 13
**Web**: www.lazaret-sete.com
**Mail**: le-lazaret@capfrance.com
**GPS coordinates**: 43°23'40.01 N 003°40'26.60 E

**ARRIVING**

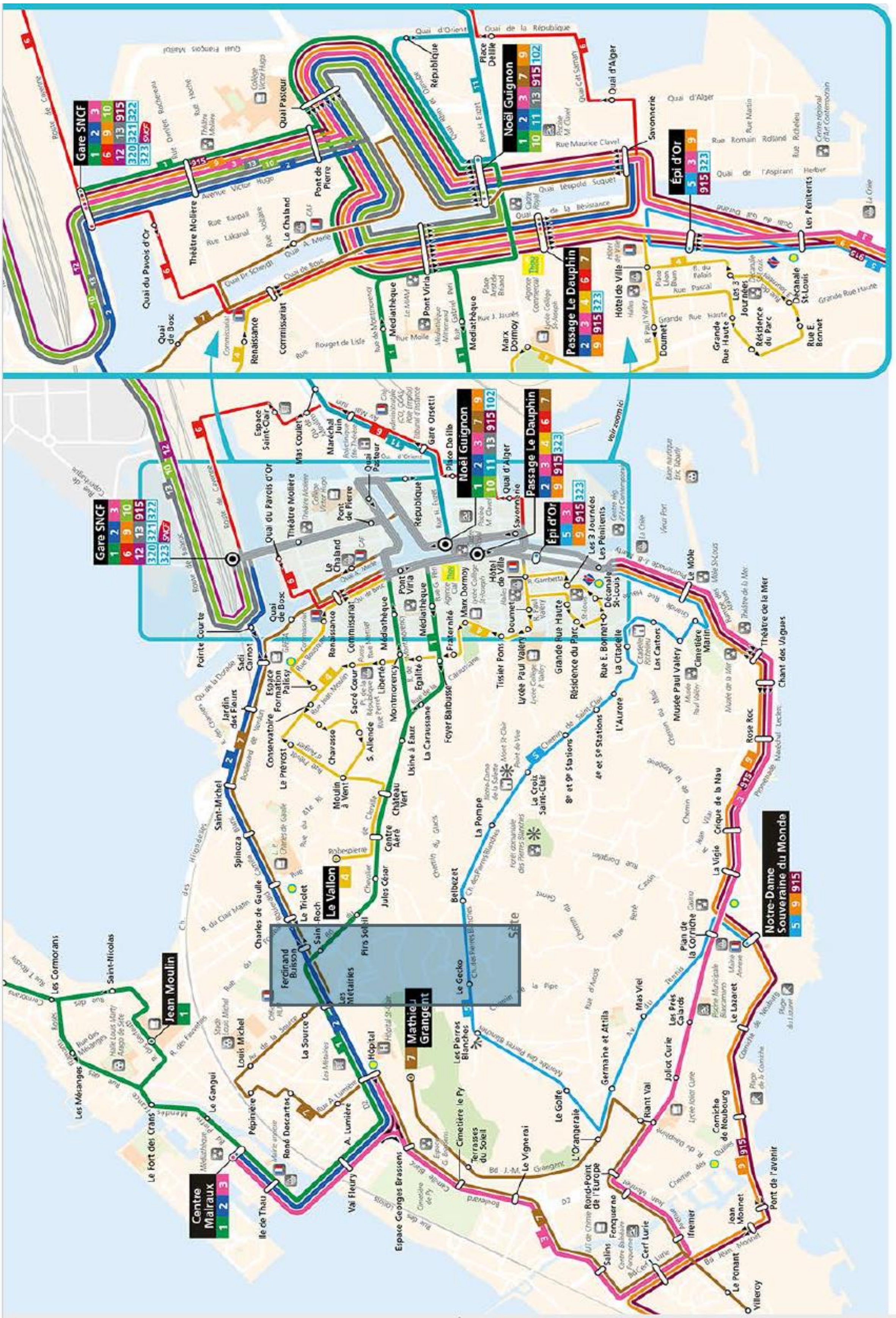
**By Air:**
Montpellier Méditerranée Airport is the closest airport. At you arrival you will find taxi. We strongly encourage you to share a taxi using the forum.
If you want to book a taxi in advance you can use
**Taxi Valenti, +33 611 57 18 05, taxi.valenti@sfr.fr**

**By train:**
In Sète, there is a SNCF railway station, which is covered by TGV. At your arrival you can find taxis and public transportation. For city bus you can take a bus of the n°3 line operating to the Centre Malraux and stop at the halt "Plan de la Corniche". Alternatively, you can take a bus of the n°9 line operating to Marseillan Plage and stop at the halt "Le Lazaret". You can check for timetables, directions and prices at the following web address: https://www.mobilite.agglopole.fr/

**By Car :**

Free parking are available at the center of "le Lazaret".

Sète
Gare de Sète
Port de Sète
Quai d'Alger
Quai de Bosc
Avenue Victor Hugo
Avenue Gilbert Martelli
Route de Cayenne
Rue de Bruxelles
Rue de Madrid
Rue de Copenhague
Avenue des Eaux Blanches
Michelot Hervé
Pointe Longue
Rue Paul Bousquet
Rue Jean Moulin
Rue Prévot d'Augier
Rue de la Révolution
Rue de la Caraussane
Rue de Belfort
Eglise Saint Joseph
OPEN SPACE Galerie
Centre Régional d'Art Contemporain
Grande Rue Haute
Chemin de Saint-Clair
Le Môle Saint-Louis
Musée Paul Valéry
Musée De La Mer
Prom. Maréchal Leclerc
Rue Jean Vilar
Crique de l'Anau (la Conque)
Rue Robespierre
Boulevard Chevalier de Clerville
Chemin du Glacis
Chemin des Pierres Blanches
Combe d'Aubagnac
Chemin des Hirondelles
Rue du Football
Rue des Cormorans
Rue des Goélands
Avenue de la Source
Rue des Roitelets
Rue des Loriots
Auchan Sète
Espace Georges Brassens
Chemin de la Pipe
Rue d'Artois
Montée des Pierres Blanches
Boulevard Jean Mathieu Grangent
Le Lazaret,223 Rue Pasteur Benoît, 34200 Sète
Casino de Sète
Boulevard Joliot Curie
Corniche de Neuburg
Plage du Lazaret
Plage de la Corniche
Chemin des Quilles
Cimetière de Py
Canal des Quilles
Route des Quatre Chansons
Chemin Château de Villeroy
Plage des Quilles
Le Dôme
D2
Plan
Satellite
Google
Données cartographiques ©2017 Google 200 m
Conditions d'utilisation
Signaler une erreur cartographique


Gare SNCF
Noël Guignon
Passage Le Dauphin
Épi d'Or
Centre Malraux
Jean Moulin
Le Vallon
Mathieu Grangent
Notre-Dame Souveraine du Monde
Sète

# MISCELLANEOUS

**Lazaret Holiday Village**

www.lazaretsete.com

Rue du Pasteur Lucien Benoît, 34200 Sète Téléphone : 04 67 53 22 47

**Tourist office**

www.tourisme-sete.com/

60, Grande Rue Mario Roustan, 34200 Sète, 04 99 04 71 71

**Public bus**

http://mobilite.thau-agglo.fr/eng, 04 67 53 01 01

The direct bus line between the Sète SNCF railway station and the Lazaret are the line 3 and 9

**Espace Georges Brassens**

http://www.espace-brassens.fr/

67 Boulevard Camille Blanc, 34200 Sète, France 04 99 04 76 26

**Musée Paul Valéry**

http://www.museepaulvalery-sete.fr/

Rue François Desnoyer, 34200 Sète, France 04 99 04 76 16

**Others activities**

https://www.tripadvisor.fr/Attractions-g660465-Activities-Sete_Herault_Occitanie.html#ATTRACTION_LIST