ION CHANNEL MODULATION SYMPOSIUM

2019

October 30-31, 2019

Venue: Royal Sonesta Hotel, Cambridge, MA

Co-hosted by:

Agenda - October 30, 2019

08.00 AM	Sign-in - Tea/Coffee - Grand Ballroom, West Tower Level 2
09.00 AM	Welcome Remarks - Thais T Johansen, Sophion Bioscience A/S and John Dunlop, Amgen
	Session 1 - Chair: Dr Andrew Marks - Columbia University
09.15 AM	Dr Isabelle Baconguis - Vollum Institute *Architecture of the proteolysis-regulated epithelial sodium channel*
09.40 AM	Professor Yasushi Okamura - Osaka University *Regulation of ion channels by distribution of phospholipids in mouse sperm*
10.05 AM	Professor Anjali Rajadhyaksha - Weill Cornell Medical College *L-type Ca^{2+} channel neural mechanisms in neuropsychiatric-related behavior*
10.30 AM	Tea/Coffee, Exhibits and Posters Session - Skyline Rooms, West Tower Level 2
	Session 2 - Chair: Professor Derek Bowie - McGill University
11.15 AM	Dr Andrew Marks - Columbia University *Structure and Function of Ryanodine Receptor in Disease of Heart and Skeletal Muscle*
11.40 AM	Dr Vera Moiseenkova-Bell - University of Pennsylvania *Molecular Mechanisms of TRPV Channels Gating Revealed by Cryo-EM*
12.05 PM	Dr Paul Colussi - TetraGenetics *Discovery of Functional Monoclonal Antibodies against K_V1.3 and KCa3.1 using the TetraExpressTM Protein Expression Platform*
12.30 PM	Lunch - Exhibits and Posters, Skyline Rooms, West Tower Level 2
	Session 3 - Chair: Professor Diane Lipscombe - Brown University
02.00 PM	Professor Al George - Northwestern University *Decrypting human ion channel variants of unknown significance*
02.25 PM	Professor Geoffrey Abbott - University of California, Irvine *Direct activation of potassium channels by neurotransmitters and ancient medicines*
02.50 PM	Dr Anna Greka - Broad Institute *Ion channel targeted therapy for a rare disease*
03.15 PM	Professor Bruce Bean - Harvard University *Strategies for selective neuronal inhibition*
03.40 PM	Reception and Poster Session - Skyline Rooms, West Tower Level 2
05.30 PM	Group Photo and Dinner, Riverfront Room, East Tower Level 2

Agenda - October 31, 2019

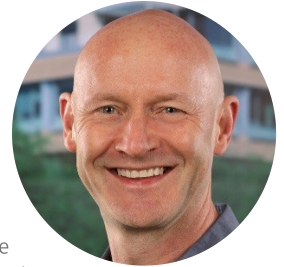
08.00 AM	Tea/Coffee - Grand Ballroom, West Tower Level 2
09.00 AM	Welcome Remarks
	Session 4 - Chair: Dr John Dunlop - Amgen
09.05 AM	Dr Yonghong Bai - Amgen *Structural basis for pharmacological modulation of the TRPC6 channel elucidated by cryo-EM*
09.30 AM	Professor Derek Bowie - McGill University *Structural underpinnings of ionotropic glutamate receptor activation*
09.55 AM	Professor Len Kaczmarek - Yale University *Regulation of cellular signaling by potassium channels*
10.20 AM	Tea/Coffee, Exhibits and Poster Session - Skyline Rooms, West Tower Level 2
	Session 5 - Chair: Dr John Dunlop - Amgen
11.00 AM	Professor Toshihisa Osaki - University of Tokyo *Artificial cell membrane platforms for functional analyses and drug screenings of ion channels*
11.25 AM	Dr Owen McManus - Q-State Biosciences *Using optogenetic assays to identify sodium channel inhibitors as pain therapeutics*
12.10 PM	Dr Saverio Gentile - University of Illinois *Potassium channel activity unveils cancer vulnerability: from signaling controlling tumor growth and metastasis to precision medicine*
12.15 PM	Lunch - Exhibits and Poster Session, Skyline Rooms, West Tower Level 2
	Session 6 - Chair: Professor Derek Bowie - McGill University
01.15 PM	Professor Diane Lipscombe - Brown University *Cell-specific control of neuronal calcium channel composition: Mechanism, Function and Disease*
01.40 PM	Dr Bryan Moyer - Amgen *Multi-modality approach to identify $Na_V1.7$ inhibitors for pain*
02.05 PM	Professor Amy Lee - University of Iowa *Voltage-gated Ca^{2+} channels of the retina: new insights into $Ca_V1.4$ channelopathies*
02.30 PM	Tea/Coffee - Grand Ballroom, West Tower Level 2
	Session 7 - Chair: Professor Diane Lipscombe - Brown University
03.10 PM	Professor Ellen Lumpkin - University of California, Berkeley *TTX-sensitive sodium channels mediate action potential firing in menthol-sensitive neurons of the dorsal root ganglion*
03.35 PM	Dr Daniel Sauter - Sophion Bioscience *Towards more physiological assays: iPSC-derived neurons tested on the 384 channel automated patch clamp platform Qube*
04.00 PM	Wrap up

Biographies - Advisory board

Derek Bowie - McGill University

Dr. Derek Bowie is the Director of the FRQS-funded research group, GEPROM, and has been a Professor at McGill University since 2002. He is the recipient of the Canada Research Chair award in Receptor Pharmacology and serves on the editorial boards of the Journal of Physiology, Current Neuropharmacology and Channels. Dr. Bowie earned his Ph.D. at the University of London after completing his undergraduate degree at Strathclyde University in Scotland. He then carried out postdoctoral training in France (Université Louis Pasteur), Switzerland (University of Zurich) and the USA (National Institutes of Health) before holding a faculty position at Emory University in Atlanta. The Bowie lab focuses on the structure-function properties of ionotropic glutamate receptors, GABA-A receptors and sodium channels as well as examining their role in neuronal circuit behaviour.

John Dunlop - Amgen



John joined Amgen in September 2016 and leads the neuroscience research program responsible for therapeutic discovery activities in neuro degenerative diseases, pain and migraine. Prior to Amgen he was leading neuroscience discovery and early development at AstraZeneca and previously held executive leadership roles in the neuroscience therapeutic area at Wyeth and Pfizer. Trained as a neuropharmacologist, John's research interests include the role of protein quality control mechanisms, innate immunity and mitochondrial dysfunction in neurodegenerative diseases such as Alzheimer's and Parkinson's disease and ALS. John was recently appointed to the HEAL (Helping End Addiction Long term) Partnership Committee, an NIH advisory committee established to support NIH initiatives launched to address the nation's opioid crisis. He is a board member of Target-ALS, a non-profit enterprise dedicated to accelerating drug discovery and development in ALS and a board observer of SiteOne Therapeutics, a biotech company focused on developing novel non-opioid pain therapeutics.

Diane Lipscombe - Brown University

Dr. Diane Lipscombe is the Director of the Carney Institute for Brain Science and Professor of Neuroscience at Brown University and current President of the Society for Neuroscience. Current research focuses on the cell-specific expression pattern, function, and pharmacology of splice isoforms of neuronal voltage-gated calcium ion channels in health and disease. In particular, the molecular mechanisms that regulate nociceptor-specific processing of neuronal calcium ion channel genes, how this process is altered after peripheral nerve injury, and its contribution to the pathophysiology of neuropathic pain.

Andrew Marks - Columbia University

Dr. Marks received his undergraduate degree from Amherst College where he was the first student in the history of the college to graduate with honors in two subjects (Biology and English), and his MD from Harvard Medical School. Following an internship and residency in internal medicine at the Massachusetts General Hospital (MGH), he was a post-doctoral fellow in molecular genetics at Harvard Medical School, and then a clinical cardiology fellow at the MGH. He is board certified in internal medicine and in cardiology. Dr. Marks is Chair and Professor of the Physiology and Cellular Biophysics Department at Columbia University. From 2002-2007 Dr. Marks was Editor-in-Chief of the Journal of Clinical Investigation. His honors include: ASCI, AAP, the National Academy of Medicine (2004), American Academy of Arts and Sciences (2005) and the National Academy of Sciences (2005). Doctor of Science Honoris Causafrom Amherst College (2009), the ASCI Stanley J. Korsmeyer Award (2010), the Pasarow Foundation Award for Cardiovascular Research (2011), the Ellison Medical Foundation Senior Scholar in Aging Award (2011), Docteur Honoris causa, de l'Université de Montpellier (2016), Glorney-Raisbeck Award from NY Academy of Medicine (2016), Columbia University Dean's Award for Excellence in promoting Diversity (2017), the Naranjan Dhalla Award for Innovative Investigators in Cardiovascular Sciences, International Academy of Cardiovascular Sciences (2018). In 2015 Dr. Marks was chosen to present the Ulf von Euler lecture at the Karolinska Institute. Dr. Marks' identification of the mechanism of action of rapamycin, inhibition of vascular smooth muscle proliferation and migration, lead to the development of the first drug-eluting stent (coated with rapamycin) for treatment of coronary artery disease. This substantially reduced the incidence of in-stent restenosis. In 2014 Dr. Marks reported the high resolution structure of the mammalian type 1 ryanodine receptor/calcium release channel (required for excitation-contraction coupling in skeletal muscle) which he had cloned and worked on to define its regulation in health and disease since 1989. His research has contributed new understandings of fundamental mechanisms that control muscle contraction, heart function, lymphocyte activation, and cognitive function. He discovered that "leaky" intracellular calcium release channels (ryanodine receptors) contribute to heart failure, fatal cardiac arrhythmias, impaired exercise capacity in muscular dystrophy, post-traumatic stress disorder (PTSD) and Alzheimer's Disease. Dr. Marks discovered a new class of small molecules (Rycals), developed in his laboratory, that target leaky ryanodine receptor channels and effectively treat cardiac arrhythmias, heart failure, muscular dystrophy and prevent stress induced cognitive dysfunction and symptoms of Alzheimer's Disease in pre-clinical studies. Rycals have shown promising results in pilot clinical trials for the treatment of heart failure and cardiac arrhythmias, and are entering clinical trials for the treatment of Duchenne Muscular Dystrophyand RyR-1 myopathy. Dr. Marks is Chair of the Scientific Advisory Board (SAB) of the RYR-1 Foundation and of ARMGO Pharma, Inc. Abio tech company focused on developing therapeutics targeting leaky RyR channels.

Biographies - Speakers

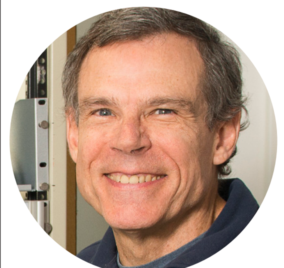
Geoffrey Abbott - University of California, Irvine

Dr. Geoffrey W. Abbott earned his Ph.D. in Biochemistry from University of London in 1997, after which he pursued his postdoctoral studies on ion channel function and disease at Yale University, supported by a Wellcome Trust Fellowship. He was made a tenured full professor at Weill Cornell Medical School in 2011 after ten years on the faculty, and soon afterwards moved to take up his present position as Professor of Physiology and Biophysics at University of California, Irvine. Funded by multiple NIH institutes, Dr. Abbott currently studies voltage-gated potassium channels, especially their interaction with and modulation by ancillary subunits, solute transporters, neurotransmitters, metabolites and new and ancient medicines.

Isabelle Baconguis - Vollum Institute

Dr. Baconguis received her BA in Biochemistry at University of Penn in 2005, and in 2007, joined the OHSU Neuroscience Graduate Program. During her doctoral research at the Vollum Institute, she studied acid sensing ion channels (ASICs), members of the superfamily of amiloride-sensitive and Na^+-selective trimeric ion channels. Using a combination of x-ray crystallography and electrophysiology, she exploited toxin-dependent modulation of ASIC function to elaborate molecular mechanisms of gating, selectivity and ion channel block. A recipient of the NIH Director's Early Independence Award, Dr. Baconguis skipped postdoctoral training and moved immediately into an independent position as the inaugural Vollum Fellow at the Vollum Institute in 2013, and was promoted to Assistant Scientist in 2016. With access to state-of-the-art electron microscopy core at OHSU, Dr. Baconguis' lab is unraveling the molecular underpinnings of whole-body salt balance to better understand diseases such as hypertension. Using the powerful technique of cryo-electron microscopy, scientists in the Baconguis lab were the first to resolve the structure of the epithelial sodium channel, an ion channel that is vital to the human body's regulation of sodium ion concentration.

Bruce Bean - Harvard University



Bruce Bean is Professor of Neurobiology at Harvard Medical School. His research interest is in the physiological function and pharmacology of ion channels in mammalian neurons, with a goal of helping discover new treatments for disorders involving altered neuronal excitability, including epilepsy and pain.

Biographies - Speakers

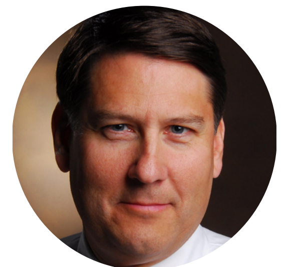
Paul Colussi - TetraGenetics Inc.

Paul has spent over 19 years in the biotech sector focusing on the development and application of recombinant protein platforms. Paul joined New England Biolabs in December of 2000 to work on developing the K. lactis yeast expression platform as well as supporting its release to the scientific community in the summer of 2005. In 2008 Paul was recruited to TetraGenetics to lead the development of the Tetrahymena thermophila expression platform and in particular its use in generating high-yields of correctly folded membrane proteins-specifically recombinant human ion channels. Since then Paul has led multiple programs with large pharma and biotech strategic partners to generate historically intractable proteins to support drug discovery programs. In recent years Paul has established TetraGenetics' proprietary drug pipeline focused on the discovery and development of mAbs targeting therapeutically important ion channels. Paul received his Bachelor of Science degree (Hons) from the University of Sydney before doing his PhD at the University of Illinois, Urbana/Champaign.

Saverio Gentile - University of Illinois

Dr. Saverio Gentile is an Assistant Professor in the Department of Medicine at the University of Illinois in Chicago. He is interested in understanding the role of ion channels in cancer biology. Dr. Gentile's group has brought to light several important functions regarding potassium channels activity that control a variety of cancer markers including proliferation, metastasis and metabolism. These works have also revealed that potassium channels can be pharmacologically targeted to develop a safe and efficient therapeutic strategy against currently untreatable breast or ovarian cancers.

Al George - Northwestern University



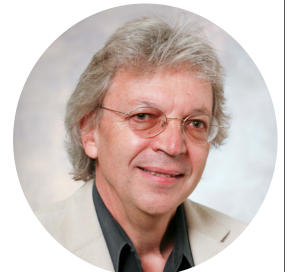
Dr. George is the Magerstadt Professor and Chair of the Department of Pharmacology, and Director of the Center for Pharmacogenomics at the Northwestern University Feinberg School of Medicine. He has been a pioneer in elucidating the genetics and pathogenesis of channelopathies with a focus on genetic disorders caused by voltage-gated ion channel mutations associated with disorders of membrane excitability including cardiac arrhythmia syndromes and epilepsy.

Biographies - Speakers

Anna Greka - Broad Institute

Anna Greka is a physician-scientist leading the translation of scientific discoveries from the laboratory to clinical trials. She is an Associate Professor at Harvard Medical School (HMS); an Associate Physician in the Renal Division in the Department of Medicine at Brigham and Women's Hospital (BWH); and the founding director of Kidney-NExT, a Center for Kidney Disease and Novel Experimental Therapeutics at BWH. Dr. Greka is also an Institute Member of the Broad Institute of MIT and Harvard, where she directs the institute's Kidney Disease Initiative (KDI) and the ion channel therapeutics interest group (CHAnnel Therapeutics, CHArT).The Greka laboratory specializes in the development of precision therapies for difficult-to-treat diseases with a special interest in genetically defined disorders. Specifically, her lab studies mechanisms of cell survival and metabolic regulation, including calcium signaling and transient receptor potential (TRP) ion channel biology. The Greka laboratory is also interested in using the modern tools of genomics and other multi-omic approaches to understand disease mechanisms, including mechanisms of disrupted cellular metabolism, with important connections to obesity and diabetes. Finally, the study of ion channel biology remains an active area of investigation, with a special focus on harnessing the considerable therapeutic potential of ion channels for a wide range of diseases, from kidney to neurologic disorders. Dr. Greka has been the recipient of several honors, including the 2018 Seldin-Smith Award for Pioneering Research from the American Society of Clinical Investigation(ASCI), a 2017 Presidential Early Career Award for Scientists and Engineers (PECASE), and a 2014 Top 10 Exceptional Research Award from the Clinical Research Council. She also serves on the Harvard-MIT M.D.-Ph.D. Program Leadership Council. Dr. Greka holds an A.B. in biology from Harvard College and an M.D. and Ph.D. in neurobiology from HMS. She received her medical and scientific training in the Harvard-MIT program in Health Sciences and Technology (HST) in the laboratory of David Clapham, MD, P.hD., where, as a Howard Hughes Medical Institute (HHMI) predoctoral fellow, she explored the role of TRP channels in neuronal growth cone motility.

Len Kaczmarek - Yale University



Len Kaczmarek is a Professor of Pharmacology and Cellular and Molecular Physiology at Yale University School of Medicine. He carried out his undergraduate and graduate work at the University of London and carried out research at the University of California at Los Angeles, the Free University of Brussels, Belgium and the California Institute of Technology before joining the Yale faculty in 1981.He served as Chairman of the Yale Department of Pharmacology from 1989 to 1998. Professor Kaczmarek's laboratory investigates the biological role of potassium channels, as well as other classes of ion channels, in neuronal function.He is currently investigating the way mutations in these proteins in humans are responsible for several forms of intellectual disability and autism. Much of his work focused on the K_V3 family of potassium channels, which are found predominantly in neurons capable of firing at high rates. His laboratory cloned and characterize the $K_v3.1b$ channel and described its modulation in intact animals. They have also recently found that the $K_v3.3$ channel directly regulates both cell death and the cortical actin cytoskeleton by binding the pro-survival protein Hax-1. In addition, the Kaczmarek laboratory cloned the genes for the Slack and Slick channels (KCNT1 and KCNT2) that underlie Na^+-activated K^+ channels (KNa channels). They found that the Slack protein interacts with the Fragile X Mental Retardation Protein (FMRP) as well as other cytoplasmic signaling molecules. Moreover, they characterized a variety of human mutations in these channels that lead to childhood seizures coupled to very severe intellectual disability. Dr. Kaczmarek has authored or edited several books and is co-author of the textbook "The Neuron".

Amy Lee – University of Iowa

Dr. Amy Lee is Professor of Molecular Physiology and Biophysics and Assistant Dean for Research of the Carver College of Medicine at the University of Iowa. Her research focuses on the functions and regulation of voltage-gated Ca^{2+} channels, particularly with respect to ribbon synapses of the retina and inner ear. She and her group have led discoveries regarding the mechanisms that fine-tune Ca^{2+}-dependent modulation of Ca_V channels, and the importance of such mechanisms for synaptic function. She has served as a Council member and Chair of the Exocytosis/Endocytosis subgroup of the Biophysical Society, and as a member of the editorial board of Molecular Pharmacology, Journal of Biological Chemistry, and Journal of General Physiology.

Biographies - Speakers

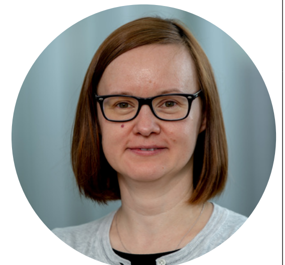
Ellen Lumpkin - University of California, Berkeley

Ellen A. Lumpkin is a Professor of Cell & Developmental Biology the University of California, Berkeley in the Department of Molecular & Cellular Biology and the Helen Wills Neuroscience Institute. She is also an Adjunct Associate Professor of physiology & cellular biophysics, an Affiliate Investigator in the Zuckerman Mind Brain Behavior Institute, and Co-Director of the Thompson Family Foundation Initiative in Chemotherapy-Induced Peripheral Neuropathy & Sensory Neuroscience at Columbia University. Lumpkin's research focuses on molecules, cells and neural signals that give rise to skin sensations such as touch, pain and itch. Dr. Lumpkin earned her BS in Animal Science from Texas Tech University and performed her PhD training in neuroscience with Dr. A. James Hudspeth at UT Southwestern Medical Center and The Rockefeller University. She completed her postdoctoral training in physiology & biophysics with Dr. Jonathon Howard at the University of Washington.

Owen McManus - Q-State Biosciences

Owen McManus, Ph.D., is a drug discovery scientist with extensive experience in drug discovery, electrophysiology, and technology development. He worked for over twenty years at Merck Research labs where he led basic research teams at multiple discovery stages including target identification and validation, assay development, lead identification and optimization. These teams produced a number of clinical candidate compounds in multiple therapeutic areas. He was also Director of Operations at the Johns Hopkins Ion Channel Center, which provided HTS screening and lead optimization for ion channel and transporter targets for the academic community through the NIH Molecular Libraries program. He also worked as Director, Ion Channel Screening and Drug Discovery at Essen Bioscience, a leading instrument development company. At both Essen and Merck, he worked on development of novel instrumentation and technologies to support drug discovery, which have led to several marketed products. He is currently Chief Technology Officer at Q-State Biosciences working to advance Q-State technologies in drug discovery efforts.

Vera Moiseenkova-Bell - University of Pennsylvania



Dr. Moiseenkova-Bell is a membrane protein biochemist and a structural biologist with expertise in cryo-electron microscopy (cryo-EM). Her research is focused on structure-function analysis of Transient Receptor Potential (TRP) channels and their interaction with agonists/antagonists to enhance our understanding of their function at the molecular level. In addition, her laboratory research program seeks to understand how TRP channels regulate cellular functions and the role of their dysregulation in human disease. After obtaining a M.S. degree in Physics from Moscow State University in 1999, Dr. Moiseenkova-Bell switched to a biological research area and received a Ph.D. in Cellular Physiology & Molecular Biophysics from the University of Texas Medical Branch in 2004. During her graduate work, she was the first to develop a methodology for overexpression and purification of functional TRP channels for structural studies. As a postdoctoral fellow, Dr. Moiseenkova-Bell continued her work on TRP channels at Baylor College of Medicine (BCM). During her training at BCM, she was the first researcher to solve and report the structure of a TRPV1 channel using cryo-EM. Because of this achievement, she received the Ruth McLean Bowman Bowers Excellence in Research Award from BCM. In 2009, Dr. Moiseenkova-Bell joined Department of Pharmacology at Case Western Reserve University (CWRU) as a tenure-track Assistant Professor and was promoted to Associate Professor with tenure in 2016. Dr. Moiseenkova-Bell moved to University of Pennsylvania in 2018, where she is continuing her work on understanding molecular mechanisms of TRP channel activation, inhibition and desensitization using cryo-EM at the Department of Systems Pharmacology and Translational Therapeutics. She is also a Faculty Director of the Beckman Center for Cryo-EM and Electron Microscopy Resource Laboratoryat the University of Pennsylvania. In the past ten years, Dr. Moiseenkova-Bell established herself as an independent scientist and as an expert in the field of TRP channels, focused on structural and functional analysis. She has published papers in Journal of Biological Chemistry, Journal of General Physiology, Molecular and Cellular Biology, Structure, Nature Communications, Nature Structural and Molecular Biology. She has given numerous invited seminars and presentations both at the national and international levels. Dr. Moiseenkova-Bell has secured funding for her research from American Lung Association, American Heart Association, Mt. Sinai Foundation, Pfizer and NIH.

Biographies - Speakers

Bryan Moyer - Amgen

Bryan Moyer, Ph.D., is a Scientific Director in the Neuroscience Department at Amgen. Dr. Moyer leads drug discovery teams to identify novel therapeutics for pain and migraine and functions as the head of the Neuroscience electrophysiology group. He has a strong background in voltage- and ligand-gated ion channel molecular/cellular biology, functional expression, pharmacology, screening, drug discovery and translational models of disease. Dr. Moyer's research leverages diverse therapeutic modalities including small molecules, peptide and antibodies to identify transformational medicines for patients. Prior to his position at Amgen, he was Associate Director, Ion Channel Biology at Senomyx, where he was responsible for the discovery, cloning, expression, and screening of novel ion channels expressed in taste receptor cells. Dr. Moyer received his Ph.D.in Physiology from Dartmouth Medical School and did post-doctoral work at the Scripps Research Institute where he studied wild-type and mutant CFTR trafficking and function in polarized epithelial cells in models of cystic fibrosis.

Yasushi Okamura - Osaka University

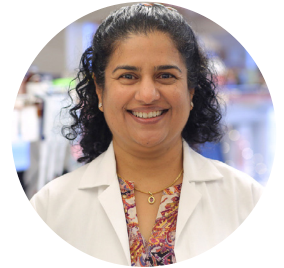
Yasushi Okamura graduated from School of Medicine at University of Tokyo and obtained license of medical doctor in 1985, and then went to the Graduate School of Medicine (PhD degree at University of Tokyo in 1989 on the theme of electrophysiology of voltage-gated ion channels). After learning molecular biology as a postdoctoral fellow in the laboratory of Dr. Gail Mandel (currently, professor of Vollum Institute) at State University of New York at Stony Brook until 1990, he came back to University of Tokyo as the lecturer to work on developmental regulation of ion channel expression until 1995. He then moved to the National Institute of Bioscience and Human-technology as the senior researcher and group leader in 1995. In 2001, he became a professor at Okazaki Institute for Integrative Bioscience where he pioneered a new research field of voltage-evoked cell signals through serendipitously discovered membrane proteins that have voltage sensor domains but not authentic pore domain. Since 2008, he has been a professor at Osaka University. His group has been working mainly on two membrane proteins. Voltage-gated proton channel, Hv1, is widely conserved from marine plankton to human, and expressed in immune cells, airway cells and sperm in human. It has the voltage sensor domain but lacks authentic pore domain. Hv1 is dimeric and each subunit has intrinsic proton-permeation pore. Voltage-sensing phosphatase, VSP, is not an ion channel but a hybrid protein consisting of the voltage sensor domain and PTEN-like phosphoinositide phosphatase. Motion of single-voltage sensor is coupled with the intrinsic PTEN-like enzyme to rapidly alter profiles of several species of phosphoinositides. Okamura's group has been studying molecular mechanisms of these proteins by combining electrophysiology, voltage clamp fluorometry, mathematical approach and genetic incorporation of fluorescent unnatural amino acid. They also aim to clarify novel cellular voltage signals both through studying knockout mice of these proteins.

Toshihisa Osaki - University of Tokyo

Toshihisa Osaki received his PhD degree in Organic and Polymeric Materials in 2002 from Tokyo Institute of Technology, Japan. He worked as a postdoctoral research fellow at Leibniz Institute of Polymer Research, Dresden, Germany (2002–2006), National Institute of Advanced Industrial Science and Technology, Japan (2006–2007), and at LIMMS/CNRS-IIS, The University of Tokyo (2007–2009). Currently, he is working at Kanagawa Institute of Industrial Science and Technology as a project assistant leader, concurrently serving as a project assistant professor at Institute of Industrial Science, The University of Tokyo. His research has focused on the development of artificial cell membrane platforms for functional analyses of ion channels with a single molecule level.

Biographies - Speakers

Anjali Rajadhyaksha – Weill Cornell Medical College



Anjali Rajadhyaksha, Ph.D., is associate professor of neuroscience at Weill Cornell Graduate School of Medical Sciences and Brain and Mind Research Institute, Weill Cornell Medicine, Cornell University in New York City, New York. Her research focuses on deciphering the molecular mechanisms underlying addiction- and neuropsychiatric-related behaviors, with a particular focus on L-type Ca^{2+} channels. Dr. Rajadhyaksha received her undergraduate training in chemistry at Bombay University, India and obtained her Ph.D. degree in molecular biology from Purdue University, West Lafayette, Indiana. She then completed her postdoctoral training in neuroscience at Massachusetts General Hospital, Harvard Medical School and thereafter joined Weill Cornell Medicine as a faculty member.

Daniel Sauter - Sophion Bioscience

Daniel Sauter received his PhD in molecular biomedicine from University of Copenhagen where he investigated the role of various ion channels in pancreatic cancer. After graduating, he worked as Research Scientist in a start-up company, Acesion Pharma where he was involved in the primary screening using automated electrophysiology.

Daniel joined Sophion Bioscience in 2016 as Application Scientist and recently relocated from the Danish headquarter to the US to manage the lab in Woburn and support customers in the North American market.

Presentation Abstracts

Direct activation of potassium channels by neurotransmitters and ancient medicines

Geoffrey Abbott
University of California, Irvine

γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in vertebrate CNS. The canonical action of GABA is via binding to neuronal GABA receptors (GABARs) to induce hyperpolarization by intrinsic ($GABA_{A/C}Rs$) or extrinsic ($GABA_BRs$) ion channel activation. Voltage-gated potassium channels KCNQ2-5, especially KCNQ2/3 heteromers, generate the neuronal M-current, another important hyperpolarizing force. Here, we discuss our recent finding that GABA and related metabolites directly activate KCNQ2/3 channels, and KCNQ2/3-dependently hyperpolarizes cells, with sensitivity comparable to the most sensitive $\alpha/\beta/\gamma$ $GABA_ARs$. We identified the M-channel GABA binding site as KCNQ3-W265, a position conserved for >500 million years in deuterostome clades but absent in protostomes and in cardiac-expressed KCNQ1. M-channel activation is a novel, unexpected mechanism for physiological and therapeutic inhibitory actions of GABA and analogues. This work has led to further discoveries in KCNQ channel pharmacology, including isolation of a potent KCNQ channel activator from cilantro. We also found that activation of the vascular-expressed KCNQ5 is a common mechanism for a variety of genetically and culturally diverse hypotensive botanical folk medicines. The implications of this work will be discussed with respect to KCNQ channel physiology, pharmacology and drug discovery.

Architecture of the proteolysis-regulated epithelial sodium channel

Isabelle Baconguis
Vollum Institute

The epithelial sodium channel (ENaC), a member of the ENaC/DEG superfamily, regulates Na^+ and water homeostasis. ENaCs assemble as heterotrimeric channels that harbor protease-sensitive domains critical for gating the channel. The structure of human ENaC in the uncleaved state determined by single-particle cryo-electron microscopy reveals that ENaC assembles with a 1:1:1 stoichiometry of $\alpha{:}\beta{:}\gamma$ subunits arranged in a counter-clockwise manner. The shape of each subunit is reminiscent of a hand holding a ball. Surrounding the ball-domain are the palm, finger, and thumb domains. The finger domain of each subunit forges interaction with the knuckle domain of the adjacent subunit. Wedged between the finger and thumb domains is the elusive protease-sensitive inhibitory domain poised to regulate conformational changes of the 'finger' and 'thumb,' domains that play critical roles ingating the ion channel.

Structural basis for pharmacological modulation of the TRPC6 channel elucidated by cryo-EM

Yonghong Bai
Amgen

Transient receptor potential canonical (TRPC) proteins form nonselective cation channels that play physiological roles in a wide variety of cells. Six TRPC isoforms, TRPC1 and TRPC3-TRPC7, are found in human. There is growing evidence supporting the therapeutic potential of TRPC6 inhibition in treating pathological cardiac and renal conditions. In particular, gain-of-function mutations of TRPC6 have been implicated in hereditary focal segmental glomerulosclerosis (FSGS), a potential cause of end stage renal disease. However, development of potent and selective small-molecule antagonists of TRPC6 is hampered by limited understanding of the molecular mechanism of TRPC6 modulation. Here we report high-resolution cryo-EM structures of the human TRPC6 channel in both antagonist-bound and agonist-bound states. The structures reveal binding modes for the small-molecule modulators corroborated by mutagenesis data. The antagonist binds to a cytoplasm-facing pocket formed by S1-S4 and the TRP helix, whereas the agonist wedges at the subunit interface between S6 and the pore helix. Conformational changes associated with ligand binding illuminate mechanisms of channel inhibition and activation. Structural analysis also suggests some FSGS-related mutations of TRPC6 increase channel activity by disrupting interfacial interactions in the cytoplasmic domain. Our results reveal principles of drug action that will guide future design of small molecules to ameliorate TRPC6-mediated diseases.

Presentation Abstracts

Strategies for selective neuronal inhibition

Bruce Bean
Harvard University

The action potential in the squid giant axon is generated by just two voltage-activated conductances, but mammalian neurons typically express well over a dozen different kinds of voltage-activated ion channels. Different types of neurons express different combinations of ion channels, underlying the wide variety of firing patterns seen in different neurons. In principle, the differential channel expression in different neurons can be exploited to develop pharmacology to differentially inhibit firing of particular cell types. Existing drugs already exploit such differential channel expression – for example, we find that phenytoin and carbamazepine more effectively inhibit action potential firing in excitatory glutamatergic neurons than in inhibitory GABAergic neurons. Increasing knowledge about differential expression levels of various channels and how currents through those channels interact to control firing of various types of neurons may allow rational strategies for drug development, although the complexity of interactions of multiple channels to control firing makes the execution of this strategy challenging.

Structural underpinnings of ionotropic glutamate receptor activation

Derek Bowie
McGill University

The Bowie Lab uses a combination of techniques to study ionotropic glutamate receptors (iGluRs), GABA-A receptors and more recently, Na^+ channels. All ion-channel families are widespread in the vertebrate brain and fulfill many important roles in healthy individuals as well as being implicated in disease states associated with postnatal development (e.g. autism, schizophrenia), cerebral insult (e.g. stroke, epilepsy) and aging disorders (e.g. Alzheimer's disease, Parkinsonism). We are looking at iGluRs, GABA-A receptors and Na^+ channels at two inter-related levels. In molecular terms, we are examining the events that occur when each ion-channel family is activated with the aim of developing novel therapeutic compounds. At the cellular level, we are studying the role that iGluRs, GABA-A receptors and Na^+ channels fulfill in shaping the behaviour of neuronal circuits and how these processes may be corrected in disease states. The talk will focus on recent findings showing how the apo state of AMPA-type iGluRs is regulated by alternative splicing of the flip/flop cassette and by auxiliary proteins. The findings have a broad implication for how other signaling proteins work as well as providing new insight into how different AMPARs signal in the brain.

Discovery of Functional Monoclonal Antibodies against $K_V1.3$ and KCa3.1 using the TetraExpress™ Protein Expression Platform

Paul Colussi
TetraGenetics

The discovery of functional antibodies targeting ion channels is a challenging endeavor that typically requires addressing low recombinant protein yield, sequence conservation among species and limited extracellular epitope target areas. To overcome these technical difficulties, TetraGenetics, Inc. has developed TetraExpress™, a protein expression platform that takes advantage of the unique biology of Tetrahymena thermophila, to allow rapid production of correctly folded and functional human ion channels. Recombinant ion channels can be isolated at >90% purity, with a yield of 0.2 -4 mg per liter of culture, and formulated in phospholipids, amphipols or on a solid support. TetraExpress™ in combination with different antibody discovery platforms was utilized to target two potassium channels, $K_V1.3$ and KCa3.1. Both channels are involved in the regulation of Ca^{2+} signaling in T cells, and they are generally recognized as therapeutic targets for autoimmune disorders as well as other indications. $K_V1.3$-specific antibodies were generated from immunized chickens and llamas, while KCa3.1-specific antibodies were identified by screening human antibody phage libraries derived from healthy (naïve) individuals and autoimmune disease-affected patients. Ten $K_V1.3$-specific and two KCa3.1-specific antibodies were found to be functional and potent (IC_{50}<10nM) in electrophysiology assays. In addition, select $K_V1.3$ and KCa3.1 antibodies inhibit proliferation of stimulated human T cells. Discovery and development of functional monoclonal antibodies against other ion channels is currently underway.

Presentation Abstracts

Potassium channel activity unveils cancer vulnerability: from signaling controlling tumor growth and metastasis to precision medicine

Saverio Gentile
University of Illinois

Decades of studies on ion channels have vastly demonstrated the critical functions of these proteins in many physiological and pathological conditions and have provided for an extraordinary pharmacopeia of useful compounds, often with selective actions and minimal side effects. Nevertheless, the role of ion channels in cancer biology is still unknown and underexplored. Our research demonstrates that different cancer cell types, e.g., breast, ovarian and melanoma express specific K^+ channels that are a key factor in cancer homeostasis. We demonstrated that the activity of specific K^+ channels can regulate critical cellular events that are hallmarks of cancer. Here we present data showing how activity of different potassium channels control biochemical pathways underlying proliferation in breast cancer or ovarian cancer. We show that pharmacological manipulation of these potassium channels arrests cancer growth in *in vitro*, *in vivo* and *ex vivo* models of breast and ovarian cancers. Our data indicate that stimulation of K^+ channel activity produces alteration of a variety of signaling ranging from changes of the cellular oxidative state to alteration of gene expression including onco-suppressors and onco-genes. Also, we show how pharmacological manipulation of K^+ channels affects cancer metastasis. We have demonstrated that pharmacological manipulation of specific K^+ channels produces a strong inhibitory effect on cancer cell motility and increases cell-cell attachment. Our data show that stimulation of K^+ channel activity affects a variety of biochemical pathways including gene expression and protein trafficking. These studies are important because they contribute to a better understanding of the role of ion channels in cancer and provide an opportunity to design a novel clinically effective and safe therapeutic strategy against cancer.

Decrypting human ion channel variants of unknown significance

Al George
Northwestern University

The explosive growth in known human gene variation presents enormous challenges to current approaches for variant classification that have implications for diagnosis and treatment of many genetic diseases. For disorders caused by mutations in cardiac ion channels as in congenital arrhythmia syndromes, *in vitro* electrophysiological evidence has high value in discriminating pathogenic from benign variants, but these data are often lacking because assays are cost-, time- and labor-intensive. We implemented a strategy for performing high throughput, functional evaluations of ion channel variants that repurposed an automated electrophysiological recording platform developed previously for drug discovery. We demonstrated success of this approach by evaluating 78 variants in KCNQ1, a major gene involved in genetic disorders of cardiac arrhythmia susceptibility. We benchmarked our results with traditional electrophysiological approaches and observed a high level of concordance. This strategy also enabled studies of dominant-negative behavior of variants exhibiting severe loss-of-function. We also investigated the functional and pharmacological properties of 70 KCNQ2 variants, a related gene involved in severe childhood-onset epilepsy. Our results illustrate an efficient and high throughput paradigm linking genotype to function for two disease-associated human ion channels that will enable data-driven classification of large numbers of variants and create new opportunities for precision medicine.

Ion Channel targeted therapy for a rare disease

Anna Greka
Broad Institute

Focal Segmental Glomerulosclerosis (FSGS) is the leading histopathology underlying progressive kidney diseases characterized by proteinuria and podocyte loss. Inherited forms of FSGS are caused by Rac1-activating mutations. In podocytes, Rac1 induces TRPC5 ion channel activity and cytoskeletal remodeling. However, it is unknown whether TRPC5 activity mediates the onset and progression of FSGS, and whether blocking this activity can provide therapeutic benefit. We identified a small molecule, AC1903 that specifically blocks TRPC5 channel activity in glomeruli of proteinuric rats. Here we demonstrate that chronic administration of AC1903 suppresses severe proteinuria and prevents podocyte loss in a transgenic rat model of FSGS. The efficacy of AC1903 was confirmed in a well-established preclinical rat model of hypertensive proteinuric kidney disease and in human kidney organoids. These data indicate that TRPC5 activity is induced to drive disease, and TRPC5 inhibitors may be valuable for the treatment of progressive kidney diseases.

Presentation Abstracts

Regulation of cellular signaling by potassium channels

Len Kaczmarek
Yale University

The greatest diversity among the over 70 different alpha subunits of potassium channels lies in their cytoplasmic N-terminal and C-terminal domains that interact with other cellular constituents. These domains allow regulation of channel activity by second messengers and protein kinases and are also required for appropriate subcellular localization of channels in neurons and other excitable cells. Recent studies have indicated that the interactions of channels with cytoplasmic signaling molecules can directly trigger cellular events that control cell survival, as well as normal physiological functions. This presentation will focus on the voltage-dependent potassium channel $K_V3.3$, which is encoded by the KCNC3 gene, and is expressed at high levels in cerebellar Purkinje cells and in neurons of the auditory brainstem, where it localizes primarily to presynaptic nerve terminals. Mutations in KCNC3 result in Spinocerebellar Ataxia type 13 (SCA13), a condition that leads to cerebellar degeneration and the inability to localize sounds in space. The cytoplasmic C-terminus of $K_V3.3$ bind Hax-1, a cell survival protein required for survival of the cerebellum. This interaction leads to the formation of a dense network of actin filaments under the plasma membrane close to presynaptic release sites. This actin network is required for endocytosis of synaptic vesicle membranes at release sites. Both fast and slow endocytosis are disrupted in neurons that either lack $K_V3.3$ or express a mutant subunit that is functional as a channel but fails to nucleate actin. Moreover, in neurons that express the mutant that fails to nucleate the underlying actin cytoskeleton, an alternative pathway of endocytosis is triggered. This pathway is activated by increased activity of TBK1 (Tank Binding Kinase-1) leading to the formation of multivesicular bodies/late endosomes that contain the Hax-1 survival protein. These mechanisms are likely to contribute to disruption of function and eventual cell death in neurons that express $K_V3.3$ mutations.

Voltage-gated Ca^{2+} channels of the retina: new insights into $Ca_V1.4$ channelopathies

Amy Lee
University of Iowa

In photoreceptor synaptic terminals, $Ca_V1.4$ L-type channels mediate Ca^{2+} signals that trigger the exocytotic release of glutamate that initiates the transmission of visual information through the retina. More than 140 mutations in the human *Cacna1f* gene encoding $Ca_V1.4$ have been identified, most of which are linked to visual disorders including congenital stationary night blindness type 2. One of the most severe visual phenotypes is linked to a gain-of function *Cacna1f* mutation causing the substitution of an isoleucine with a threonine in a pore-lining region of domain IIS6 (I745T). How this mutation leads to visual impairment is poorly understood. Here we show that in addition to causing a very large hyperpolarizing shift in voltage-dependent activation, I745T greatly slows deactivation of $Ca_V1.4$. These effects of I745T are amplified in a naturally occurring $Ca_V1.4$ splice variant lacking a portion of a distal C-terminal modulatory domain. I745T also modifies the ion selectivity of $Ca_V1.4$ in a manner that depends on the identity of the auxiliary $\alpha_2\delta$ subunit. In a knock-in I745T mutant mouse, photoreceptor synapses are poorly maintained, and synaptic transmission is greatly impaired. We conclude that I745T modifies the pore of $Ca_V1.4$ in ways that stabilize channel opening and weaken ion selectivity, both of which may be detrimental to the stability and function of the first synapse in the visual pathway.

Cell-specific control of neuronal calcium channel composition: Mechanism, Function and Disease

Diane Lipscombe
Brown University

Alternative splicing is a form of RNA processing that is critical for virtually every stage in the life cycle of a neuron – starting from early neuronal differentiation, to axonal guidance and synapse formation, to supporting cell signaling and plasticity, and for programmed cell death. A number of neurologic and psychiatric diseases are linked to abnormal alternative splicing and therapeutic strategies to correct or compensate for these types of pathogenic mutations in neurons are in clinical use. We have studied normal and pathological changes in the pattern and the mechanisms that regulate alternative splicing of voltage-gated calcium ion channel genes. We identified RNA and DNA binding proteins that combine to regulate the composition of voltage-gated calcium ion channel expression in a cell-specific pattern through control of pre mRNA splicing. The precise pattern of alternative splicing has major functional impact on calcium ion channel properties including biophysics, G protein inhibition, and sensitivity to morphine. Calcium channels in *Trpv1*-lineage nociceptors are strongly inhibited by mu-opioid receptor inhibition because these cells express a unique splice isoform of $Ca_V2.2$ that has high sensitivity to Galpha-dependent inhibition. Cell-specific splicing of $Ca_V2.2$ pre mRNA is disrupted in *Trpv1*-lineage nociceptors following peripheral nerve injury, and this is associated with reduced mu-opioid receptor inhibition and effectiveness of morphine as an analgesic. We now show that the unique $Ca_V2.2$ mRNA exon composition in *Trpv1*-lineage nociceptors is regulated by cell-specific, hypomethylation of CpG sites in the alternatively spliced exon in the Cacna1b gene. The methylation state of the target *Cacna1b* exon regulates protein binding to this exon impacting exon splicing. We show that exon-specific methylation levels are increased following nerve injury leading to disruption of the normal pattern of RNA splicing in *Trpv1*-lineage nociceptors, and subsequent reduction in the sensitivity of the calcium channel to mu-opioid receptor activation. Pathological disruption of DNA methylation in the *Cacna1b* following nerve injury changes alternative splicing resulting in reduced morphine efficacy. Supported by NIH grant NS055251

Presentation Abstracts

TTX-sensitive sodium channels mediate action potential firing in menthol-sensitive neurons of the dorsal root ganglion

Ellen Lumpkin
University of California, Berkeley

Dorsal root ganglion (DRG) neurons encode somatosensory modalities including thermoreception, nociception and itch. My group recently identified a requirement for tetrodotoxin-sensitive sodium channels in action potential firing in menthol-sensitive neurons, which mediate cooling and noxious cold sensations. By contrast with most small-diameter DRG neurons, menthol-sensitive neurons exhibited robust ongoing discharges at room temperature *in vitro*. To support this heightened excitability, menthol-sensitive neurons displayed depolarized membrane potentials, lower firing thresholds, and higher evoked firing frequencies compared with menthol-insensitive neurons. Biophysical and pharmacological studies demonstrated distinct functional contributions of Na_V subunits, with $Na_V1.1$ driving action potentials in menthol-sensitive neurons, whereas other small-diameter neurons employ TTX-resistant Na_V channels. As menthol is a analgesic and anti-pruritic, these findings suggest $Na_V1.1$ in the peripheral nervous system as a target for sensory disorders.

Structure and Function of Ryanodine Receptor in Disease of Heart and Skeletal Muscle

Andrew Marks
Columbia University

Ryanodine receptors (RyRs) are ubiquitous intracellular calcium (Ca^{2+}) release channels required for the function of many organs including heart and skeletal muscle, brain, pancreatic beta cell, and vascular tone. In disease, defective function of RyRs due either to stress (hyperadrenergic and/or oxidative overload) or genetic mutations can render the channels leaky to Ca^{2+} and promote heart failure, muscular dystrophy, diabetes mellitus, and neurodegenerative diseases. RyRs are massive structures comprising the largest known ion channel macromolecular complex exceeding 3 million Daltons. RyRs mediate the rapid release of Ca^{2+} from the endoplasmic/sarcoplasmic reticulum (ER/SR) to stimulate cellular functions including cardiac and skeletal muscle contraction. Recent advances in single-particle cryogenic electron microscopy (cryo-EM) have enabled the determination of atomic-level structures for RyR. These structures (now at 2.6Å) have illuminated the mechanisms by which RyR channels function and are regulated by ligands. In this presentation the structure, function, gating and activation mechanisms of RyRs in normal and disease states are discussed.

Using optogenetic assays to identify sodium channel inhibitors as pain therapeutics

Owen McManus
Q-State Biosciences

More than 100M people in the US suffer from chronic pain. Current treatments, including opioids and non-steroidal inflammatory agents, have severe limitations for chronic treatment due to tolerance and dose-limiting toxicities. To develop novel pain therapeutics, we have created an all-optical electrophysiology (Optopatch) screening platform using engineered optogenetic proteins. Blue and red light can be used to stimulate and record bioelectrical activity, respectively. For target-based screening application, we demonstrate Optopatch measurements in engineered excitable HEK cells (spiking HEK cells) with heterologous expression of Na_V channels targets implicated in pain transmission, including $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$. The Na_V channel Optopatch assays can distinguish compounds with different working mechanisms (state-dependent versus state independent block). To achieve a throughput of 10,000 compounds/day, we developed a next generation kinetic plate reader (SWARM) capable of recording 24 wells simultaneously. We have executed a 14,000-compound screen on $Na_V1.7$ spiking HEK cells and identified novel $Na_V1.7$ inhibitors with sub-micromolar potency. To further qualify candidate compounds, we have developed proprietary secondary assays for hit prioritization, which combined the Optopatch platform with an *in vitro* model of chronic pain, in which dorsal root ganglion (DRG) sensory neurons are exposed to a mixture of inflammatory mediators. This assay leverages our custom Firefly microscope to make highly-parallelized measurements of DRG excitability, where ~100 neurons can be measured in parallel with single-cell precision and ms-temporal resolution. The newly identified hits are benchmarked against $Na_V1.7$ inhibitors in Phase II trials, and selected hits demonstrate favorable profiles in our secondary assays. Interestingly, we found that highly selective $Na_V1.7$ cannot fully reverse the inflammatory mediator-induced DRG hyperexcitability; we dissect Na_V-subtype contributions to hyperexcitability using control pharmacological probes and protein knockdown with antisense oligonucleotides (ASOs).

Presentation Abstracts

Molecular Mechanisms of TRPV Channels Gating Revealed by Cryo-EM

Vera Moiseenkova-Bell
University of Pennsylvania

The transient receptor potential vanilloid 5 (TRPV5) channel is a highly calcium selective ion channel that regulates systemic calcium homeostasis by acting as a critical gate for calcium reabsorption in the kidney. Human polymorphisms of this channel have exemplified the importance of TRPV5 in disorders of calcium homeostasis, but atomic level information regarding this channel had remained unknown. To address this gap, we utilized cryo-electron microscopy (cryo-EM) to understand the gating and modulation of TRPV5. Moreover, this newly obtained structural information coupled with structure-based virtual screening allowed us to uncover novel inhibitors of TRPV5 and revealed unique mechanisms of TRPV5 inhibition. These studies have thus expanded the base of understanding of TRPV5 at the atomic level and laid the foundation for future rational drug design.

Multi-modality approach to identify $Na_V1.7$ inhibitors for pain

Bryan Moyer
Amgen

The voltage-gated sodium channel $Na_V1.7$, encoded by the SCN9A gene, is being aggressively pursued as a target for novel, non-additive pain therapeutics based on learnings from human genetics and channel function in action potential firing in dorsal root ganglion (DRG) nociceptor neurons. Industry exploration of pharmacological modulators of channel function encompass chemotypes engaging different binding pockets and blocking various channel gating states including sulfonamides, pore blockers (tetrodotoxin and saxitoxin), peptides (from spider venoms) and local anesthetics (e.g. lidocaine). Major challenges for successful $Na_V1.7$ drug discovery include isoform selectivity, the need to block action potential firing in nociceptors and biodistribution of large molecule therapeutics, including peptides and biologics, to axons behind the blood nerve barrier. Examples will be provided for drug discovery efforts utilizing peptides, peptide-antibody conjugates, sulfonamides as well as siRNA. Venom screens and structure-activity relationship analyses of tarantula-derived peptides identified potent and selective $Na_V1.7$ inhibitors that were 1000x selective over $Na_V1.5$ and $Na_V1.4$ and that blocked action potential firing in both DRG neurons as well as C-fibers in an *ex vivo* skin nerve preparation. Conjugation of peptides to carrier antibodies enabled half-life extension in pharmacokinetic studies as well as biodistribution to axons in nerve fascicles. Sulfonamide antagonists have been optimized with 100-1000x selectivity over other human Na_V isoforms. These compounds block action potential firing in human DRG neurons and block mouse pain behavior in multiple translatable *in vivo* models, demonstrating target access and engagement. Ongoing work by the industry to prosecute potent and selective $Na_V1.7$ inhibitors in clinical trials will ultimately test the hypothesis that acute pharmacological blockade of $Na_V1.7$ can decrease human pain.

Regulation of ion channels by distribution of phospholipids in mouse sperm

Yasushi Okamura
Osaka University

Voltage-sensing phosphatase, VSP, consists of a voltage sensor domain similar to that of voltage-gated ion channels and a cytoplasmic PTEN-like phosphoinositide phosphatase region. Voltage sensor motion induced by membrane depolarization leads to phosphatase activity of dephosphorylating PI(4,5)P2 through tight coupling to the enzyme region. VSP gene is highly conserved from marine invertebrates to human. However, physiological function of VSP still remains unclear in any animal species so far. Across animal species, VSP is expressed in testis. We have recently found that VSP knockout mice showed abnormal sperm function as represented by circular swimming pattern of matured sperm. Mass spectrometry analysis of knockout mice sperm showed increased PI(4,5)P2and reduced PI(4)P, indicating that VSP is constitutively active and dephosphorylates PI(4,5)P2 in sperm. We also performed freeze fracture EM method to visualize individual free PI(4,5)P2 molecule in membrane to find that distribution of PI(4,5)P2 is not homogenous in mouse sperm and such heterogeneity was disrupted in sperm of VSP gene knockout animal. Sperm motility upon capacitation was abnormal in knockout animal, indicating that VSP plays role in regulating sperm motility through biased phosphoinositide distribution in sperm tail. These findings raise a new concept of regulation by phosphoinositide gradient.

Presentation Abstracts

Artificial cell membrane platforms for functional analyses and drug screenings of ion channels

Toshihisa Osaki
University of Tokyo

Electrophysiological monitoring provides detailed characteristics of ion channels for development of candidate drug molecules. The automated patch-clamp systems drastically improved the data throughput on the whole-cell electrophysiological assays, as the throughput was severe disadvantage of the conventional technique using manual patch clamping. Here, we introduce a multichannel electrophysiological platform based on a planar lipid bilayer, aiming for automated and high-throughput assay of ion channels with the single protein level. We take advantage of a simple procedure of a bilayer formation at the interface between a pair of aqueous droplets submerged in lipid-dispersed oil. The single component of the chip consists of a pair of mm-sized wells to settle the droplets and a thin separator to confine the area of the interfacial bilayer between the droplets. Ion channels of interest, extracted from cells or expressed by *in-vitro* protein synthesis, are incorporated into the bilayer. The ionic current through the ion channels were obtained with a pair of Ag/AgCl electrodes embedded at the bottom of each well. Using the platform, we demonstrated electrical recordings of ion channels from plasma membrane (hBK, Kir2.1, ASIC1, and TRPV1) and organelle membranes (RyR and TRPML1), and screening assays of modulators on the ion channels.

L-type Ca^{2+}channel neural mechanisms in neuropsychiatric-related behavior

Anjali Rajadhyaksha
Weill Cornell Medical College

Treating neuropsychiatric disorders remains a challenge. Understanding the brain circuit and biological mechanisms that underlie neuropsychiatric-associated symptoms will likely provide important insights on how brain function goes awry in these disorders. Recent genetic studies have identified CACNA1C that codes for the $Ca_V1.2$ isoform of L-type calcium channels, as a candidate risk gene for a variety of neuropsychiatric disorders, including bipolar disorder, schizophrenia and major depression, suggesting a contribution of calcium signaling mechanisms in the pathophysiology of these disorders. This is not surprising given that L-type calcium channels play a key role in brain development and neuronal plasticity. Additionally these disorders are highly co-morbid with substance abuse. Using animal models, we have been interested in identifying how deficiency in $Ca_V1.2$ channels can impact signaling mechanisms and neural circuits that underlie neuropsychiatric-related behaviors. Our ultimate goal is to integrate findings from altered gene function to behavior to provide a platform to explore therapeutic interventions.

Towards more physiological assays: iPSC-derived neurons tested on the 384 channel automated patch clamp platform Qube

Daniel Sauter
Sophion Bioscience

Human induced pluripotent stem cells (hiPSCs) can be differentiated into multiple cell types, including neurons and cardiomyocytes. This allows to establish novel, highly predictive human disease models in vitro. Ion channels represent a class of highly attractive therapeutic targets in the nervous and the cardiovascular system, rendering electrophysiological studies of hiPSCs important for their usage in drug discovery. However, such studies have traditionally been limited by the labor-intensive and low throughput nature of the manual patch clamp technique. In the present study, we used our automated, 384 channel patch clamp system Qube to develop robust assays for the study of hiPSC-derived neurons. Three different hiPSC-based neuronal disease models were investigated. These hiPSC-derived neurons were generated from patients presenting either spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). In a first step, voltage-clamp was used to isolate and characterize individual endogenous currents and compare these to currents recorded from cells derived from healthy individuals. Both the SMA and ALS disease model exhibited elevated voltage-gated sodium channel (Na_V) currents. We confirmed that the isogenic control cells of both models reverse the observed increase in Na_V current. Finally, we tested a set of reference compounds, targeting various ion channels, on all three disease models and evaluated the assay with regard to stability and reproducibility. Our recordings demonstrate the feasibility of assaying hiPSC-derived neurons on the APC platforms Qube. Altogether, these results can facilitate evaluating the use of hiPSC for early drug development and in extension personal medicine.

Poster Abstracts

Novel first-in-class voltage-gated calcium channel positive allosteric modulators that stabilize the open state of the channel and have therapeutic potential for a variety of neuromuscular conditions

Professor Stephen D. Meriney
University of Pittsburgh

Poster board 01

We have recently developed a series of novel, first-in-class,voltage-gated calcium channel (VGCC) positive allosteric gating modifiers that are selective for the types of voltage-gated calcium channels that regulate transmitter release at neuromuscular synapses (Ca_V2). These small molecules do not cross the blood-brain barrier well and have been shown to have therapeutic potential to treat a variety of neuromuscular weakness conditions and diseases (Lambert-Eaton myasthenic syndrome (LEMS), Spinal Muscular Atrophy (SMA), the MUSK form of myasthenia gravis (MUSK-MG), presynaptic congenital myasthenic syndromes, normal aging-induced dynapenia (weakness associated with frailty), and significant adverse effects from BOTOX (onabotulinumtoxin A) treatment).We have characterized VGCC modulation several ways. First, we have characterized the *in vitro* VGCC modulator activity of these new molecules using patch clamp recordings of calcium channel current in HEK293 cells and shown that they better access the closed state, but then stabilize the open state of the channel (to increase mean open time). Second, we have generated *in silico* homology models of $Ca_V2.1$ in open and closed states and identified candidate drug binding sites in domain III, which we have then validated using *in vitro* site-directed mutagenesis and patch clamp recordings of drug effects on calcium current. Third, we have used disease model mice to show that our drugs can enhance transmitter release from the neuromuscular junction and improve motor performance on behavioral tasks *in vivo*. Because these drugs can only modify the channel after channel opening, and speed of modification (which varies for each drug analog) is on the order of milliseconds, during a brief action potential depolarization, only a fraction of VGCCs will be modified. As might be expected, these drugs are more effective when the action potential is of longer duration because a greater number of VGCC will be opened. As a consequence, our drugs work synergistically with the clinically relevant potassium channel blocker 3,4 diaminopyridine (DAP) to greatly enhance transmitter release at neuromuscular synapses. In mouse models of neuromuscular disease, a combination of our novel VGCC gating modifier with DAP completely reverses neuromuscular weakness. Lastly, these novel VGCC modulators represent a new class of experimental compounds for the study of VGCC gating and modulation.

$GABA_A$ receptor pharmacology: Rat hippocampal astrocytes and HEK293 cells stably expressing $GABA_A(\alpha_5\beta_3\gamma_2)$ investigated on an automated patch clamp setup (QPatch)

Weifeng Yu
Sophion Bioscience

Poster board 02

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nerve system (CNS) and is exerting its effect by binding to GABA receptors. The importance of GABA in the CNS is underscored by the devastating consequences of dysfunctional GABA signaling. Consequently, pharmacological manipulation of $GABA_A$ has a large therapeutic potential. High throughput platforms are hence desirable to assess compound effects on GABA receptors and the patch clamp technique remains the gold standard. In the present study, the 48-channel automated patch clamp platform QPatch and QPatch II was used to develop assays that are amenable for drug development.

A thorough pharmacological characterization is presented both using a single $GABA_A$ receptor clone (α5β3γ2), stably expressed in a HEK293 cells and a heterogeneous GABA receptor population endogenously expressed in cultured primary hippocampal astrocytes.

In $GABA_A(\alpha_5\beta_3\gamma_2)$-HEK293 cells, 100μM GABA elicited a 1.98 ± 0.64 nA response (peak current) per cell and the EC_{50} value was 4.9 μM ($CI_{95\%}$: 4.5 to 5.2 μM). This current was both bicuculine and picrotoxin sensitive and was potentiated by diazepam. Astrocytes displayed a 70 ± 38 pA current amplitude when exposed to 3mM GABA. The EC_{50} value was 161 μM ($CI_{95\%}$: 91.2-287μM) and the current was completely blocked by picrotoxin.

This study demonstrates that it is indeed possible to perform high throughput electrophysiological evaluation of $GABA_A$ receptor pharmacology in both stably expressing cells and primary astrocytes using the automated patch clamp platform, QPatch and QPatch II.

iPSC-derived motor neurons on the automated patch clamp platforms Qube and QPatch

Daniel Sauter
Sophion Bioscience

Poster board 03

Human induced pluripotent stem cells (hiPSCs) can be differentiated into multiple cell types, including neurons and cardiomyocytes. This gives rise to a novel way of establishing human disease models, which in turn can be used for drug development in vitro. Ion channels represent highly attractive therapeutic targets in the nervous and the cardiovascular systems, rendering electrophysiological studies of hiPSCs important for their usage in drug discovery. However, such studies have traditionally been limited by the labor-intensive and low-throughput nature of patch-clamp electrophysiology. Here we use our automated patch clamp systems Qube 384 and QPatch 48 in order to increase throughput and reduce time lines.

Our observations include channel expression versus time in culture, the pharmacological dissection of endogenous ion channels (e.g. Na_V and K_V), identification of ligand-gated receptors, and recordings of action potentials using the current clamp feature. Also, we show the electrophysiology of a spinal muscular atrophy (SMA) and an amyotrophic lateral sclerosis (ALS) model. The disease model for SMA was derived by mutations in the SMN1 gene and shows enhanced sodium channel activity but no shift in the normalized current voltage relationship. ALS was here mimicked by a single point mutation in the superoxide dismutase 1 protein (SOD1), D90A, which had previously been identified in recessive, dominant and seemingly sporadic pedigrees. Cells carrying this point mutation displayed larger sodium currents, which eventually led to neurofilament aggregation, neurite degeneration and other phenotypes. We could confirm that the electrophysiological effect could be reversed by point mutation to D90D.

Our measurements validate the feasibility of measuring hiPSC ion channel currents using the APC platforms Qube and QPatch. Altogether, these results can facilitate evaluating the use of hiPSC for early drug development and in extension personal medicine.

Biophysical and pharmacological profiling of multiple voltage-gated sodium channel subtypes on QPatch II

Sung Hoon Park
Sophion Bioscience

Poster board 04

Voltage-gated sodium channels (VGSC) are responsible for the initiation and propagation of action potentials in excitable cells. VGSC have been identified as excellent drug targets for treatment of pain, epilepsy and other neurological disorders. Early compounds, however, were developed using empirical approaches. The identification of the molecular identity of VGSC in combination with technological advances, such as the automated patch clamp technique, provide the basis for a rational design of subtype-selective compounds.

To date, 9 functional mammalian isoforms (Na_V1.1–1.9) have been described in the literature. The various subtypes differ in their expression pattern and exhibit distinct biophysical and pharmacological profiles. All have in common that they produce a transient inward current in response to membrane depolarization. During this process the VGSC transitions from a closed to and open into an inactivated state. Interestingly, inhibitor compounds often exhibit different pharmacological profiles dependent upon the ion channel conformational state.

In the present study, the second generation QPatch (QPatch II; Sophion Bioscience) was used in combination with adaptive voltage protocols to investigate state-dependent inhibition of tetrodotoxin (TTX) and tetracaine on 8 different VGSC subtypes (Na_V1.1-8). A first step was to determine the half inactivation potential $V_{½}$ (inactivation) for each individual cell. This value was then used during the next steps as preconditioning pulse. Such an adaptive protocol allowed to determine IC_{50} values for both the closed and the inactivated state and reduce heterogeneity of the cells. Both IC_{50} values and biophysical parameters of the different subtypes align well with literature values.

Poster Abstracts

Piezo2 integrates mechanical and thermal cues in vertebrate mechano-receptors

Yury A. Nikolaev Yale University	Poster board 05

The detection of mechanical touch and temperature is essential for interaction with the physical world. Here, we report that cold potentiates the transformation of mechanical touch into ionic current in cutaneous mechanoreceptors from different vertebrate animals. We found that cold increases the peak amplitude of mechanically activated current in all subtypes of mechanoreceptor sensory neurons in mice and ducks. We show that this process is mediated by the mechanosensitive ion channel Piezo2, the principal detector of touch in somatosensory neurons. This effect can be replicated in heterologous systems. In contrast, in HEK293T cells, cold inhibited mechanically activated current of Piezo1, supporting the idea that this channel is highly sensitive to changes in the physical properties of the plasma membrane. By swapping the membrane-embedded blade domains between Piezo2 and Piezo1, we demonstrate that the blade domains are essential for cold-induced potentiation of Piezo2. Together, these results reveal that somatosensory neurons can directly integrate thermal and mechanical stimuli via Piezo2, and that such integration is dependent on the blade domains of the channel.

Zheng, W., Nikolaev, Y.A., Gracheva, E.O. and Bagriantsev, S.N., 2019. Piezo2 integrates mechanical and thermal cues in vertebrate mechanoreceptors. Proceedings of the National Academy of Sciences, 116(35), pp.17547-17555.

Validation of B'SYS K_V3.x cell lines on Sophion QPatch 48 HTX

Ellen Braksator B'SYS	Poster board 06

K_V3.x receptors have recently emerged as potential targets for treatment of a variety of CNS disorders, including epilepsy, ataxias, hearing disorders, schizophrenia and cognitive impairments. $K_v3.1$–$K_v3.4$ protein isoforms contribute to the high-frequency firing of neurons such as auditory brain stem neurons, fast-spiking cortical and hippocampal GABAergic interneurons, and Purkinje cells of the cerebellum, and play an important role in the regulation of intrinsic excitability and neurotransmitter release at presynaptic terminals of many neurons. B'SYS has generated a panel of cell lines stably transfected with $K_V3.1a/b$, $K_V3.2$, $K_V3.3$ and $K_V3.4$ subunits. Pharmacological characterization of these cell lines was performed on the Sophion QPatch 48 HTX, with a first preliminary set of K_V3.x modulators including the selective serotonin reuptake inhibitor fluoxetine and the small molecule $K_V3.1$ and $K_V3.2$ positive modulator AUT00206. In addition, the modulation of these channels by the protein kinases PKA and PKC was investigated. The current-voltage (IV) relationship of activation and concentration-dependent drug effects (Hill slope and EC_{50}/IC_{50}values) will be presented. These cell lines are useful tools for the drug discovery industry for the screening of compounds against K_V3.x channels.

Characterization of *in vitro* pharmacology of CMPI; a photo-reactive nicotinic receptor potentiator with subunit stoichiometry selectivity

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Pharmacological targeting of neuronal nicotinic acetylcholine receptors (nAChRs) holds promise in the development of drug strategies to alleviate chronic pain, treat nicotine dependence, and slow cognitive decline associated with Alzheimer's disease. Despite their potential clinical applications, there are only few neuronal nAChR drugs available clinically. Drugs that enhance nAChRs include agonists that directly activate nAChRs and positive allosteric modulators (PAMs) which enhance ACh potency and/or efficacy by binding at sites distinct from the ACh binding sites. There is an increasing interest in the development of nAChR PAMs because they enhance ACh signaling without alteration of cholinergic transmission due to the prolonged activation and desensitization of nAChRs seen with agonists. Several nAChR PAMs have been developed at Amgen Inc. via chemical modification of substituted piperidine structure including 3-(2-chlorophenyl)-5-(5-methyl-1-(piperidin-4-yl)-1H-pyrrazol-4-yl)isoxazole)(CMPI) (Albrecht et al. 2008; Springer et al. 2008). At submicromolar concentrations, CPMI potentiated α4β2 nAChR but not any other major nAChR subtype (Albrecht et al 2008). Our studies have established CMPI as a nAChR PAM that potentiates the low-sensitivity (α4)3(β2)2 nAChR but not the high-sensitivity (α4)2(β2)3 nAChR (Hamouda et al. 2016) and identified the binding site for CMPI within the extracellular domain at the α4:α4 subunit interface which exists only in the (α4)3(β2)2 nAChR subtype (Wang et al., 2017). The effect of CMPI on ACh responses of (α4)3(β2)2 nAChR was characterized by enhancement of ACh potency by ~100 fold with no significant effect on the efficacy of ACh (Wang et al., 2017). In addition, using *Torpedo* nAChR as a model, we have established CMPI as a photoreactive PAM possessing broad amino acid side chain reactivity (Hamouda et al. 2016). Overall, these results establish CMPI as one of the most selective nAChR PAMs identified to date.

Poster Abstracts

Optimization of TRPC5 Channel Inhibitors Via High Throughput Electrophysiology Compound Screening

Juan L. Pablo
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Poster board 08

Kidney disease is a widespread and debilitating condition that offers little by way of specific and targeted therapy. Inhibition of the ion channel TRPC5 has been shown to be protective in a variety of kidney disease models. Our group has recently developed a TRPC5 inhibitor, AC1903, that has an IC_{50} <10 μM but unlike other reported inhibitors does not block the highly related TRPC4 or TRPC6 channels in the same concentration range. Although a promising first step, there remains a need to develop compounds with better drug-like properties. To address this, we have developed a high throughput electrophysiology assay that has enabled the screening of a library of compounds synthesized in an iterative fashion based on known inhibitors of TRPC5. Here we present the results of a structure-activity study that has allowed us to identify compounds with improved potency while retaining specificity.

siRNA Knockdown of SCN9A Expression: Correlation of mRNA, Protein and $Na_V1.7$ Currents in Heterologous Cells

Bryan D. Moyer
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Poster board 09

$Na_V1.7$ represents a compelling target for non-addictive pain therapeutics due to its exquisite human genetic validation with both loss of function and gain of function pain disorders. Multiple modalities have been pursued in the development of $Na_V1.7$ inhibitors including small molecules (local anesthetics, sulfonamides and saxitoxin analogs), peptides/peptide-Ab conjugates derived from tarantula venoms as well as antibodies. We evaluated the potential of siRNA as a new modality for $Na_V1.7$ inhibition in stably transfected HEK293-human $Na_V1.7$ cells. Starting with 20 commercially available siRNA sequences, 6 showed greater than 2-fold specific reduction of SCN9A expression compared to C911 controls using a digital droplet RT-PCR assay. The siRNA yielding the largest reduction in transcript levels was further evaluated in dose-response studies following delivery by electroporation on the Maxcyte STX platform, correlating three endpoints: (1) SCN9A transcript levels, (2) $Na_V1.7$ protein levels using a specific antibody in Western blotting, and (3) $Na_V1.7$ current levels measured using an automated electrophysiology assay on the IonWorks Barracuda platform with a resting/closed state voltage protocol. Results from these studies demonstrated that:

- Reduction of SCN9A mRNA or $Na_V1.7$ protein by 50% did not impact $Na_V1.7$ current levels
- Reduction of SCN9A mRNA or $Na_V1.7$ protein by 75-85% was necessary to reduce $Na_V1.7$ current levels by 50%
- Robust reduction of $Na_V1.7$ current levels by 95% necessitated ~90% reduction of SCN9A mRNA and protein levels

In summary, high levels of siRNA-mediated knockdown of SCN9A transcripts are required for inhibition of $Na_V1.7$ currents in heterologous HEK293 cells. Future studies will inform the degree of siRNA-mediated SCN9A transcript knockdown required to inhibit endogenous $Na_V1.7$ currents in a native dorsal root ganglia neuron background.

Crystal structures of human glycine receptor α3 bound to a novel class of analgesic potentiators

Xin Huang
Amgen

Poster board 09

Current therapies to treat persistent pain and neuropathic pain are limited by poor efficacy, side effects and risk of addiction. Here, we present a novel class of potent selective, central nervous system (CNS)-penetrant potentiators of glycine receptors (GlyRs), ligand-gated ion channels expressed in the CNS. AM-1488 increased the response to exogenous glycine in mouse spinal cord and significantly reversed mechanical allodynia induced by nerve injury in a mouse model of neuropathic pain. We obtained an X-ray crystal structure of human homopentameric GlyRα3 in complex with AM-3607, a potentiator of the same class with increased potency, and the agonist glycine, at 2.6-Å resolution. AM-3607 binds a novel allosteric site between subunits, which is adjacent to the orthosteric site where glycine binds. Our results provide new insights into the potentiation of cysteine-loop receptors by positive allosteric modulators and hold promise in structure-based design of GlyR modulators for the treatment of neuropathic pain.

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