The 1st Joint Symposium on Connexin Channel Biophysics: Current Directions and Future Opportunities

The Book of Abstracts

March 19, 2026 • Online

A specialized meeting on the latest advances in intercellular communication.

The 1st Joint Symposium on Connexin Channel Biophysics: Current Directions and Future Opportunities

Jointly organized by the Lithuanian University of Health Sciences (LSMU), the University of California, Davis (UC Davis), and the Kaunas University of Technology (KTU).

Organizing Committee

- Prof. Jorge E. Contreras, University of California, Davis, Department of Physiology and Membrane Biology
- Dr. Lina Kraujalienė, Lithuanian University of Health Sciences, Institute of Cardiology
- Assoc. Prof. Tadas Kraujalis, Lithuanian University of Health Sciences, Institute of Cardiology; Kaunas University of Technology, Department of Applied Informatics
- Assoc. Prof. Mindaugas Šnipas, Lithuanian University of Health Sciences, Institute of Cardiology; Kaunas University of Technology, Department of Mathematical Modelling

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Welcome Message

It is our great pleasure to welcome researchers, clinicians, and students to this inaugural event dedicated to the study of connexin channels and gap junction biophysics. This symposium provides a platform for the exchange of new ideas, the development of collaborations, and discussions of recent advances in our understanding of intercellular communication.

This meeting reflects the strength of international and interdisciplinary collaboration. It is jointly organized by the Lithuanian University of Health Sciences (LSMU), the University of California, Davis (UC Davis), and the Kaunas University of Technology (KTU), with speakers representing four continents.

The program highlights the diversity of current research—ranging from high-resolution structural insights obtained by cryo-EM to studies exploring the physiological roles of hemichannels and gap junction channels in the circulation and the heart. We hope that the *current directions* discussed during this symposium will inspire the *future opportunities* that drive the next advances in connexin research.

Thank you for joining us and for your contribution to this scientific community. We wish you a productive and inspiring symposium.

The Organizing Committee

Symposium Program

Times are shown in Pacific Daylight Time (PDT) and Eastern European Time (EET).

Session 1

- **8:00 – 8:10 PDT (17:00 – 17:10 EET)**: **Welcoming and Opening Remarks – Jorge E. Contreras,** Department of Physiology and Membrane Biology, University of California, Davis, Davis, CA, USA.
- **8:10 – 8:50 PDT (17:10 – 17:50 EET)**: **Keynote – Pablo S. Gaete**, Department of Physiology and Membrane Biology, University of California, Davis, Davis, CA, USA. *"When a Channel Behaves Like a Transporter: A Multiple-Step Mechanism for Molecular Flux in Cx26"*.
- **8:50 – 9:10 PDT (17:50 – 18:10 EET)**: **Ananth Prasad Burada**, Molecular Biophysics Unit, Indian Institute of Science, Bengaluru, India. *"Calcium-Mediated Docking Mechanism of Connexin-32 Revealed by Cryo-EM Structural and Functional Analysis"*.
- **9:10 – 9:30 PDT (18:10 – 18:30 EET)**: **Lina Kraujalienė**, Laboratory of Intercellular Communication, Institute of Cardiology, Lithuanian University of Health Sciences, Kaunas, Lithuania. *"Effect of General Anesthetic Propofol on Cardiovascular Gap Junction Channels"*.
- **9:30 – 9:50 PDT (18:30 – 18:50 EET)**: **Vytautas Raškevičius**, Laboratory of Cell Culture, Institute of Cardiology, Lithuanian University of Health Sciences, Kaunas, Lithuania. *"Short Terpenes Preferentially Target Cx43 Gap Junctions Rather than Cardiac Sodium and Calcium Channels: Applications in Cardiac Ephaptic Transmission Studies"*.
- **9:50 – 10:00 PDT (18:50 – 19:00 EET)**: **BREAK**

Session 2

- **10:00 – 10:20 PDT (19:00 – 19:20 EET)**: **Pia C. Burboa**, Department of Pharmacology, Physiology and Neuroscience, Rutgers-New Jersey Medical School, Newark, NJ, U.S.A. *"Role of Endothelial S-Nitrosylated Cx43 Hemichannels in the Microcirculation"*.
- **10:20 – 10:40 PDT (19:20 – 19:40 EET)**: **Isaac E. García**, Laboratory of Molecular Physiology and Biophysics, Facultad de Odontología, Universidad de Valparaíso, Valparaíso, Chile; Instituto Milenio Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile. *"Mechanism of Gain-of-Function in Aberrant Heteromeric Hemichannels Formed by Cx43 and Cx26S17F KID-Related Mutation"*.
- **10:40 – 11:00 PDT (19:40 – 20:00 EET)**: **Lukas Gudaitis**, Laboratory of Intercellular Communication, Institute of Cardiology, Lithuanian University of Health Sciences, Kaunas, Lithuania. *"N-Terminal Amino Acids and Their Effect on Biophysical Properties of Connexin-36 Gap Junction Channels"*.
- **11:00 – 11:20 PDT (20:00 – 20:20 EET)**: **Jonathan Quan**, Department of Physiology and Membrane Biology, University of California, Davis, Davis, California, USA. *"Connexin 43 S-Nitrosylation Drives β-Adrenergic Enhancement of Cardiac Conduction and Contractility"*.

Abstracts

When a channel behaves like a transporter: A multiple-step mechanism for molecular flux in Cx26

Pablo S. Gaete[1], Deepak Kumar[2], Cynthia I. Fernandez[1], Juan M. Valdez Capuccino[3], Yu Liu[3], Andrew L. Harris[3], Yun L. Luo[2], Jorge E. Contreras[1]

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Connexin hemichannels were the first eukaryotic large-pore channels discovered and are often assumed to behave as passive aqueous conduits in which ions and signaling molecules permeate in parallel through the same open pore. We overturn this view and show that connexin 26 (Cx26) operates as a hybrid channel/transporter in which ionic conduction and small-molecule transport are mechanistically separable. We expressed Cx26 in Xenopus oocytes and paired two-electrode voltage clamp with real-time uptake of fluorescent probes to track electrical and molecular flux simultaneously. Whereas ionic currents report the canonical “channel” function, molecular transport displays transporter-like behavior: it is selective, saturable at micromolar concentrations, and competitively inhibited, implying that permeating molecules engage discrete interaction sites. Strikingly, human disease–linked Cx26 variants that lack measurable ionic currents still support near–wild-type molecular transport kinetics, revealing that electrical silence does not necessarily mean loss of molecular signaling. Molecular transport is favored at negative membrane potentials and suppressed by depolarization, opposite to expectations from hemichannel opening. We identified a conserved extracellular vestibular pocket enriched in acidic residues that transiently traps cationic molecules near the pore entrance. Depolarization stabilizes this trapped state and prevents translocation, whereas hyperpolarization promotes release and the passage to a second intracellular binding domain formed by N-terminal (NT) residues. Consistent with a two-step mechanism, vestibular mutations weaken trapping and flatten voltage dependence, whereas NT-domain mutations control selectivity and reshape the kinetics of translocation. Together, these results redefine Cx26 as a molecular machine that implements binding, selectivity, saturation, and voltage-tuned dwell times within a channel architecture, enabling regulated molecular signaling independently of ion conduction.

Calcium-Mediated Docking Mechanism of Connexin-32 Revealed by Cryo-EM Structural and Functional Analysis

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Connexin 32 (Cx32), predominantly expressed in the peripheral nervous system and hepatocytes, plays a critical role in intercellular communication through the exchange of metabolic intermediates and ions essential for cellular survival and homeostasis. These connexin channels form direct conduits between adjacent cells by assembling into gap junction channels that facilitate intercellular exchange. In myelinating Schwann cells, Cx32 forms a radial diffusion pathway across the multi layered myelin sheath, enabling the transfer of nutrients and ions. Mutations in Cx32 disrupt gap junction communication, leading to demyelination and the onset of peripheral neuropathies such as Charcot-Marie-Tooth disease. Although several structural studies of Cx32 have been reported, the molecular mechanisms underlying channel docking and gap junction conduction remain incompletely understood. In this study, we demonstrate that calcium enhances gap junction channel docking compared to conditions lacking calcium. Using cryo-electron microscopy, we solved the structure of Cx32 in the presence of calcium at 3 Å resolution and identified calcium-binding sites located within the extracellular loops that coordinate hemichannel docking. Substitution of key coordinating residue with alanine or valine significantly reduced transjunctional currents, supporting a critical role for calcium in the docking mechanism of Cx32. Structural analysis of the alanine mutant revealed the presence of hemichannels without fully formed gap junction channels, consistent with the observed reduction in intercellular conductance by dual whole cell patch clamp method. Additionally, we resolved the structure of the intracellular loop of Cx32 and are currently investigating its role in channel gating. Together, our findings provide structural and functional insights into calcium-mediated regulation of Cx32 gap junction assembly and communication.

Effect of General Anesthetic Propofol on Cardiovascular Gap Junction Channels

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Propofol is among the most widely used anesthetics for surgical anesthesia and procedural sedation. Despite its rapid onset and favorable recovery profile, the most concerning side effect is a reduction in systemic vascular resistance, which can lead to hypotension and, in severe cases, to bradycardia. Conversely, some research suggests that propofol may exert cardioprotective effects against ventricular arrhythmias and renal ischemia-reperfusion injury. Elucidating the precise mechanisms underlying these effects is essential to understand the duality of propofol's impact on cardiovascular system and preventing adverse outcomes.

In this study, we investigated the effects of propofol on Cx37, Cx40, Cx43, and Cx45 – key connexins that form gap junctions in cardiac and vascular systems. Our results demonstrate that Cx37 is the most sensitive to propofol (EC_{50} = 5.75 μM), while Cx45 is the least sensitive (EC_{50} = 44.44 μM). Cx40 and Cx43 exhibited intermediate sensitivities, with EC_{50} values of 13.96 μM and 21.04 μM, respectively. Analysis of V_j-gating parameters indicates that gating modification is not the primary mechanism for propofol-induced changes in conductance. Instead, our findings suggest that propofol reduces Cx43-mediated coupling by activating protein kinase C, which phosphorylates Cx43 and impairs intercellular communication. Other studied cardiovascular connexins do not share the same mechanism of action in response to propofol. Notably, propofol also inhibited gap junctions formed by C-truncated Cx43 variant, suggesting a complex inhibitory mechanism involving both direct and indirect pathways.

Short Terpenes Preferentially Target Cx43 Gap Junctions Rather than Cardiac Sodium and Calcium Channels: Applications in Cardiac Ephaptic Transmission Studies

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Essential oils from plants show diverse biological activities, with their primary components being terpenes, which are constructed from isoprene units. In prior work, we identified several terpenes as novel inhibitors of gap junction (GJ) intercellular communication. We also found that the inhibitory effect of the monoterpene α-pinene depended on phosphorylation of connexin 43 (Cx43). In this study, we used molecular modeling to select terpenes with varying numbers of isoprene subunits and ring structures. Patch-clamp experiments assessed their ability to inhibit Cx43 GJ conductance. The tested terpenes blocked conductance with IC_{50} values ranging from 2.6 to 38 µM. Sesquiterpenes (but not monoterpenes) showed a prolonged delay in GJ conductance recovery after washout. In addition, sesquiterpenes inhibited membrane Na^+ and L-type Ca^{2+} currents in human atrial myocytes with over 10-fold lower potency compared to their GJ effects. These distinct properties prompted us to use sesquiterpenes to test the controversial concept of ephaptic excitation in the heart. We performed microelectrode recordings and optical mapping in Langendorff-perfused rabbit hearts. Even at high doses of farnesene, higher than those sufficient to block GJ communication, ventricular electrical excitation remained intact. Only minor changes occurred in action potential parameters, consistent with farnesene's modest effects on Na^+ and Ca^{2+} currents. These results indicate that GJ inhibition does not disrupt cardiac impulse propagation as expected, suggesting that ephaptic transmission likely plays a significant role in maintaining the spread of excitation in the heart.

Role of Endothelial S-nitrosylated Cx43 Hemichannels in the Microcirculation

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Endothelial calcium (Ca^{2+}) signaling is central to microvascular regulation, governing both vasomotor tone and vascular hyperpermeability. Accordingly, defining the Ca^{2+}-permeable channels that shape endothelial responses is essential. Although connexins are classically known for forming gap junctions, emerging evidence highlights their function as undocked hemichannels (connexin hemichannels, Cx-HCs), which permit Ca^{2+} entry and may contribute to arteriolar tone regulation and inflammatory hyperpermeability. Based on this, we asked whether endothelial Cx43 hemichannels (Cx43-HCs) operate as a canonical Ca^{2+}-permeable signaling mechanism across distinct microvascular segments, including postcapillary venules.

In endothelial cells from resistance arteries, Cx43 and TRPV4 were found in close proximity. In primary resistance artery EC cultures, TRPV4 activation with GSK1016790A enhanced eNOS activity, increased NO production, and opened Cx43-HCs through S-nitrosylation, leading to Ca^{2+}-dependent activation of KCa channels and membrane hyperpolarization.

In human microvascular endothelial cells (HMVECs), Cx43 and eNOS co-localized at the membrane under basal conditions but redistributed to the cytosol following inflammatory stimulation. This relocalization was prevented by Gap19 (a selective Cx43-HC inhibitor), β-cyclodextrin (β-CD), or BAPTA-AM. In mice with impaired Cx43-HC S-nitrosylation or activity, inflammation disrupted Cx43 and eNOS organization, whereas eNOS-deficient ECs showed reduced Cx43–Cav-1 proximity. Across all mutant models, hyperpermeability responses were markedly blunted in vitro and in vivo.

These findings identify S-nitrosylation-dependent activation of Cx43 hemichannels as a unifying endothelial signaling mechanism, revealing segment-specific pathways that regulate vasomotor tone in resistance arteries and govern inflammatory hyperpermeability in postcapillary venules.

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Mechanism of Gain-of-Function in Aberrant Heteromeric Hemichannels Formed by Cx43 and Cx26S17F KID-Related Mutation

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Keratitis-ichthyosis-deafness syndrome (KID) is a clinical triad caused by syndromic mutations in connexin26 (Cx26). We have demonstrated that syndromic mutations located at the N-terminus of Cx26 allow interaction with WTCx26 and with WTCx43 forming hyperactive aberrant heteromeric hemichannels, but non-functional gap junction channels. Interestingly, oocytes co-expressing S17F and Cx26 exhibits: i) currents larger than WTCx26 ii) increased calcium apparent affinity, iii) and most remarkably a deactivation time constant value that doubles that of WTCx26. Aberrant hemichannels formed by WTCx43 and Cx26S17F showed large currents, increased calcium apparent affinity and large relaxation time constant suggesting stabilization of the open state in hemichannels. These results show that heteromeric connexin hemichannels containing human mutations in the N-terminus exhibit altered voltage and calcium regulation when co-expressed with the WTCx26 or WTCx43, and strongly suggest that aberrant heteromeric hemichannels are the main responsible for the syndromic phenotype elicited by KID related mutations in Cx26.

N-Terminal Amino Acids and Their Effect on Biophysical Properties of Connexin-36 Gap Junction Channels

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Connexin-36 (Cx36) is the predominantly expressed connexin protein in mammalian neurons where it forms electrical synapses. Cx36 gap junction (GJ) channels are also found among pancreatic β-cells; they help modulate insulin secretion. Cx36 channels are also well known for their unique biophysical properties: very low unitary conductance, low percentage of functioning channels within the GJ channel plaque, low sensitivity to transjunctional voltage (V_j), inability to form heterotypic connexin GJ channels.

Our earlier studies have showed that Mg^{2+} ions uniquely and robustly modulate wild-type Cx36 GJ channel coupling and sensitivity to V_j, and the single amino acid substitution mutations at N-terminus strongly affect these interactions. In this follow-up study, by using electrophysiological and modeling techniques, we showed that these substitution mutations also intrinsically alter V_j-gating properties and it is not just the effect of Mg^{2+} binding. Furthermore, we report that V_j-gating polarity of wild-type Cx36 of N-terminus is positive; negatively charged glutamic acid at 3rd position is crucial for determining this biophysical channel property – the substitution to neutral glutamine at this position reverses the N-terminus V_j-gating polarity of mutated Cx36*E3Q channels. Other amino acid substitutions at 8th, 13th and 18th positions (mutations E8Q, A13K, H18K, respectively) do not change the V_j-gating polarity of mutated channels, but affect electrostatic energy profiles (e.g. Cx36*E3Q channels exhibit the most pronounced shift from a negatively charged profile, Cx36*H18K channels were more similar to wild-type Cx36 channels), sensitivities and kinetics to V_j (e.g. Cx36*H18K channels exhibited the most robust changes in V_j-gating properties).

Connexin 43 S-Nitrosylation Drives β-Adrenergic Enhancement of Cardiac Conduction and Contractility

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Sympathetic activation during exercise or emotional stress enhances cardiac performance and is thought to promote electrical coupling between cardiomyocytes, which strengthens myocardial contraction. Electrical coupling in the heart is mediated by gap junction channels (GJCs) formed by connexin proteins, located at the intercalated discs between cardiomyocytes. Connexin 43 (Cx43) is the predominant connexin in the ventricular myocardium and is essential for action potential propagation. However, the mechanisms regulating Cx43 function during sympathetic activation remain poorly understood.

Recent studies show that β-adrenergic stimulation, which mimics sympathetic drive, induces nitric oxide (NO) production and S-nitrosylation of cardiac proteins, including Cx43. We found that Cx43 at the intercalated discs becomes highly S-nitrosylated following β-adrenergic stimulation, and that NO enhances Cx43 mediated coupling in a heterologous expression system. Based on these findings, we hypothesize that S-nitrosylation of Cx43 enhances electrical coupling between cardiomyocytes and contributes to increased cardiac contractility.

To test this hypothesis, we identified the Cx43 S-nitrosylation site and created a knockin mouse in which cysteine 271 is replaced by serine (C271S), then characterized these mice using telemetric electrocardiography (ECG), *ex vivo* optical mapping, and echocardiography. We found that mice harboring the C271S mutation displayed normal Cx43 expression and subcellular localization, but undetectable Cx43 S-nitrosylation. Under basal conditions, C271S mice exhibit no differences from WT mice in arrhythmogenesis or ECG intervals, but β-adrenergic stimulation with isoproterenol reveals QRS prolongation. Importantly, after isoproterenol treatment, C271S hearts fail to augment conduction velocity and contractility to the same degree as WT hearts. Together, these findings indicate that Cx43 S-nitrosylation is an important sympathetic regulatory modification that enhances GJC coupling and cardiac function.