BIOGRAPHICAL SKETCH

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NAME: Lee, Sun Joo

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POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Handong University, Gyungbuk, South Korea	Bachelor in Biological Engineering	03/96 –02/00	Cell/Molecular Biology, Food Engineering
Huntington College, Montgomery, AL		08/98 –12/98	Biology
Gwangju Institute of Science and Technology (GIST), Gwangju, South Korea	MS	03/00 –02/02	Identification of differentially expressed protein during mesenchymal cell development
Washington University, St. Louis, MO	Ph. D.	08/04 –12/10	Molecular modeling of biomembranes using multiscale molecular dynamic simulations

A. Personal Statement

My research efforts are focused on understanding the molecular basis of ion channel function. I have many years of experience and a list of publications that illustrate my expertise in the structure/function study of both prokaryotic and eukaryotic inwardly rectifying potassium (Kir) channel families. These efforts, through combined computational modeling, in vitro functional assays of purified proteins in synthetic membranes, and high-resolution (2.0 Å) crystal structure determination, have led to elucidation of the molecular mechanisms of bulk anionic lipids (PL(-)s) positive allostery in Kir 2 channel activation.

K_{ATP} channels control cell excitability in response to the cellular metabolic state and are widely expressed in multiple tissues with various subunit compositions. Inhibitors specific for vascular KATP (vK_{ATP}) channels have yet to be developed, leaving the medical needs for conditions caused by an elevated vK_{ATP} activity unmet. Specificity is critical to prevent these from affecting pancreatic KATP (pKATP) channels, as their inhibition leads to insulin hypersecretion and, consequently, hyperglycemia, a dangerous condition.

The overarching goal of the proposed research is to develop small molecule inhibitors that are more specific to vKATP channels for clinical applications. To achieve this goal, we aim to elucidate the structural basis of a few small molecule drugs, including VU0542270 (VU270), for their preferential inhibitory effect on vKATP over pKATP by directly comparing the complex structures between the two proteins using single-particle cryo-EM. With this newly acquired information, we will then optimize each of these drugs for improved efficacy and specificity. This research could facilitate clinical interventions while minimizing the risk of dangerous adverse effects, particularly hypoglycemia.

B. Positions, Scientific Appointments, and Honors

08/2018 – 06/2021 Instructor, Department of Cell Biology and Physiology, Washington University in St. Louis

07/2021 - Assistant Professor, Department of Cell Biology and Physiology,

Washington University in St. Louis

Professional Service:

05/2013 – 05/2014	Interdisciplinary 'Membrane Protein Club' Seminar Organizer
02/2020	Co-chair of the session "*Platform: Ligand-gated Channels"* at the Biophysical Society 64th Annual Meeting in San Diego, California
08/2020 -	Peer Reviewer for various journals including JGP, Science, and Comm. Biol.

Teaching Experience:

09/1999 – 12/1999	Teaching Assistant, Department of Bioscience and Food technology, Handong University, South Korea
09/2005 – 12/2005	Teaching Assistant, Department of Chemistry, Washington University in St. Louis
05/2012 - 06/2021	Advising undergraduate/graduate students joining and rotating in Nichols Lab

Awards and Scholarships

2013	Travel Award, Gordon Research Conference, South Hadely, MA
2013 – 2014	Postdoctoral fellowship, American Heart Association
2015	Postdoctoral fellowship, American Heart Association

Professional Organizations

11/2009 –	Member, Biophysical Society
06/2012 –	Member, American Heart Association
08/2018 -	Member, Society of General Physiology

C. Contributions to Science

1. Kir2 channel structure and function Membrane lipids actively regulate activities of the embedded proteins through diverse mechanisms. One of those is through direct binding and functioning as ligands. Phosphosinositol-4,5-bisphosphate (PIP_2) activation of Kir channels was shown to be positively regulated by bulk anionic lipids abundant in cell plasma membranes. Through docking simulations of various lipid headgroups, I identified a distinct site responsible for bulk anionic lipid binding, which was strongly supported by mutagenesis as well as in vitro functional assays and high resolution crystal structures determined in the PL(-) bound pre-activated state. The studies showed that PL(-) interaction pulled the CTD towards the membrane and led the protein in a high PIP_2 affinity state[1].

In order to obtain an open conformation, a negatively charged amino acid (I178D) was introduced at the gate of Kir2.2 channel, with which the mutant channel was constitutively active without PIP_2. The mutant crystal structure determined in complex with PIP_2 was slightly more open but still essentially in a closed state. In collaboration with Dr. Anna Stary-Weinzinger, this structure was placed in a membrane freed from crystallographic contacts in silico, and analyzed by molecular dynamics. The protein spontaneously further open and allowed the movement of K^+ ions throughout the whole conduction pathway. This was the first simulation to show K^+ ion movement through the channel, and even though there is significant under-sampling, the maximum conductance level observed in silico was very close to experimental measurements.

A intriguing gating propoerty of long-lived multiple sub-conductance states appeared in the mutant (I178D) protein, which is not observed in the wild type proteins. Excised-patch electrophysiology of the mutant revealed that the sub-state occupancy is titrable by pH, suggesting individual Asp residues are subject to protonation, leading to changes in the conductance level. The observation that protonation-driven conductance changes were further supported by the studies with tandem constructs varying the number of ionizable Asp residues per tetramer. These studies revealed a significant effect of protonation and deprotonation at the gate on both conduction and gating. Together with two other corresponding authors, my role in this study was to guide the first authors in designing experiments and simulations, and to interpret the results to uncover the underlying mechanisms.

a. **Lee, S-J** *et al.* Secondary anionic phospholipid binding site and gating mechanism in Kir2.1 inward rectifier channels. *Nat. Comm.* **4**, (2013).
b. **Lee, S-J** *et al.* Structural basis of control of inward rectifier Kir2 channel gating by bulk anionic phospholipids. *J. Gen. Physiol.* **148**, 227-237, (2016).
c. Zangerl-Plessl EM*, **Lee, S-J*** et al. Atomistic details of channel opening and conduction in eukarytoic Kir2.2 inward rectifier potassium channel. *J. Gen. Physiol.(2020)* **152** (1):e201912422

d. Maksaev G*, Bründl-Jirout M*, Stary-Weinzinger A, Zangerl-Plessl EM†, **Lee, S-J**, and Nichols CG†. Subunit gating resulting from individual protonation events in Kir2 channels. *Nat. Comm.* (2023) **14**, 4538

2. Anomalous Kir3.2 channels upon oxidation Oxidative stress is implicated in a variety of pathological conditions and has a direct regulatory effect on Kir channels. However, most studies on this topic have been conducted using cell-based functional assays, where quantitative control of oxidation is challenging. I took advantage of my toolkit to study G protein-activated Kir3.2 channel function through in vitro reconstitution and determined how oxidation leads to anomalous channel properties. These properties include a rapid loss of PIP2 activation and a surprisingly high ligand-independent activity by slow kinetics. In vitro assays combined with mutagenesis enabled the separation of these mixed effects, identifying two distinct oxidation events on two different cysteine residues were responsible for the two anomalous channel properties that emerged upon extended oxidation. Clearly, the approach developed in this study is applicable to study of other Kir channel regulation by oxidative stress.

a. Lee SJ†, Shoji Maeda, Jian Gao, and Nichols CG. Reversed GIRK2 gating by PIP2 upon oxidation. *Function* (2023) 4 (3): zqad016

3. Structural dynamics of Kir channels revealed by FRET assays Even though structures determined through x-ray crystallography, NMR, or single particle cryo-EM have immensely advanced our understanding of protein structure/function, a few or at most several snapshots of a protein are inevitably underrepresenting the reality of a highly dynamic entity. Fluorescence resonance energy transfer (FRET) measurements among FRET pairs located in many different parts of a protein generate a distance matrix, from which conformational changes can be inferred. I have contributed to generate structural models to visualize the inferred conformational changes during channel opening of prokaryotic KirBac1.1 channels[2]. Single molecule FRET traces reveal not only conformational changes but also kinectics of those changes. With this single molecule approach, we were able to reveal an interesting phenomenon that potassium channel selectivity filter (SF) was very dynamic, which was heavily dependent on ions occupying the SF. My role was to assess the channel function in vitro under the influence of different ions so that structure and function can be compared. The manuscript is submitted to Nature Chemical Biology with the title, 'Conformational dynamics of the KirBac1.1 potassium channel selectivity filter region revealed by single-molecule FRET'. Lipid dependent dynamics of eukaryotic Kir channels are one of my long-term questions, and this approach will be applied to understand Kir4.2 channel structural dynamics and function in the future.

a. Wang, S, **Lee S-J** *et al.* Structural rearrangements underlying ligand-gating in Kir channels. *Nat Commun* **3**, 617, (2012).
b. Wang S, **Lee S-J**, Maksaev G, Fang X, Zuo C, and Nichols CG. Potassium channel selectivity filter dynamics revealed by single-molecule FRET. *Nat. Chem. Biol.*(2019) **15**(4), 377-383

4. Atomistic details of a nanoparticle cargo delivery mechanism Nanoparticles are invented to enable target specific drug delivery and hence devised to be multifunctional for cargo loading and ligand binding for specificity. Perfluorocarbon (PFO) based nanoparticles are nano-scale emulsions stabilized by an emulsifying phospholipid monolayer surrounding hydrophobic core made of PFO molecules. The molecular level understanding how these PFO-nanoparticles interact with cell membranes and how drug molecules get transferred to cells are important to further optimize the nanoparticles in a cell-type and drug-type specific manner. I revealed the atomistic details of these interactions through multi-scale in silico modeling[3-5] and suggested the shape of PFO molecules and types of lipids to increase cargo loading capacity as well as to facilitate the nanoparticle fusion with cell membranes.

a. **Lee, S-J** *et al.* Characterization of perfluorooctylbromide-based nanoemulsion particles using atomistic molecular dynamics simulations. *J Phys Chem B* **114**, 10086-10096, (2010).
b. **Lee, S-J** *et al.* Interaction of melittin peptides with perfluorocarbon nanoemulsion particles. *J Phys Chem B* **115**, 15271-15279, (2011).
c. **Lee, S-J** *et al.* Simulation of fusion-mediated nanoemulsion interactions with model lipid bilayers. *Soft Matter* **8**, 3024-3035, (2012).

Peer reviewed publications-most relevant to the current application

1. Wang S, **Lee S-J**, Heymann S, Enkvetchakul D and Nichols CG. Structural rearrangements underlying ligand-gating in Kir channels. *Nature Communications.* (2012) **3**, 617 (PMID: 22233627)
2. D'Avanzo N, **Lee S-J**, Cheng WWL, Nichols CG. Energetics and location of phosphoinositide binding in human Kir2.1. *J. Biol. Chem* (2013) **288**, 16726-16737 (PMID: 23564459)
3. **Lee S-J**, Wang S, Borschel W, Heyman S, Goyre J, Nichols CG. Unique anionic phospholipids binding site and gating mechanism in Kir2.1 inward rectifier channels*. Nature Communications* (2013) **4**, 2786 (PMID: 24270915)
4. Zubcevic L, Wang S, Bavro V, **Lee S-J**, Nichols CG, and Tucker S. Modular Design of Ion Selectivity in a Novel Family of Prokaryotic 'Inward-Rectifier' (NirBac) Channels. *Scientific Reports* (2015) **5**, 15305
5. Cooper P, Sala-Rabanal M, **Lee S-J**, and Nichols CG. Differential Mechanisms of Cantu Syndrome-Associated Gain of Function Mutations in the ABCC9 (SUR2) subunit of the KATP channel. *J. Gen. Physiol.* (2015) **146**(60): 527
6. **Lee S-J**, Ren F, Zangerl-Plessl EM, Heyman S, Stary-Weinzinger A, Yuan P, and Nichols CG. Structural basis of bulk anionic phospholipid allosteric regulation of inward rectifier Kir2 channel gating. *J. Gen. Physiol.* (2016) **148**(6), 527-540
7. Borschel W, Wang S, **Lee S-J**, and Nichols CG. Control of Kir Channel Gating by Cytoplasmic Domain Interface (CD-I) Interactions. *J. Gen. Physiol.(2017)* https://doi.org/10.1085/jgp.201611719
8. Nichols CG and **Lee S-J**. Polyamines and potassium channels: A twenty five year romance. J. Biol. Chem (2018) doi: 10.1074/jbc.TM118.003344
9. Wang S, **Lee S-J**, Maksaev G, Fang X, Zuo C, and Nichols CG. Potassium channel selectivity filter dynamics revealed by single-molecule FRET. *Nat. Chem. Biol.*(2019) **15**(4), 377-383
10. Zangerl-Plessl EM*****, **Lee S-J*** et al. Atomistic details of channel opening and conduction in eukarytoic Kir2.2 inward rectifier potassium channel. *J. Gen. Physiol.(2020)* **152** (1):e201912422
11. **Lee SJ**†, Shoji Maeda, Jian Gao, and Nichols CG. Reversed GIRK2 gating by PIP2 upon oxidation. *Function* (2023) **4** (3): zqad016
12. Maksaev G*, Bründl-Jirout M*, Stary-Weinzinger A, Zangerl-Plessl EM†, **Lee S-J**, and Colin G. Nichols†. Subunit gating resulting from individual protonation events in Kir2 channels. *Nat. Comm.* (2023) **14**, 4538

Invited Lectures

1. **Lee S-J**. Membrane lipid regulation of potassium ion channels. U of Vienna. Austria. 2016. Talk
2. **Lee S-J.** Atomistic details of gating and conduction in a eukaryotic Kir channel. 72nd Annual Symposium of Society of General Physiology. Woods Hole, MA. 2018. Short Talk
3. **Lee S-J**. The molecular mechanisms of cholesterol regulation of Kir channels revealed by direct and quantitative approaches. Annual Biophysical Society Meeting. San Diego, CA. 2020. Platform Talk
4. **Lee SJ.** Ligand Gating of Potassium Channels. Dept. of Pharm. SIUE. 2022. Talk
5. **Lee SJ.** Ligand Gating of Potassium Channels. IQB Colloquium. South Korea. 2023. Talk
6. **Lee SJ.** Ligand Gating of Potassium Channels. Dept of Pharm. U of Vienna. Austria. 2024. Talk

D. Research Support

Completed Research Support

13POST14660069 Postdoctoral Fellowship, American Heart Association Lee (PI) 01/13 – 12/14
"Understanding of synergistic phophatidyl-4,5-bisphosphate and anionic lipids regulation of inwardly rectifying potassium (Kir) channels" **Award**: $90,772.00

15POST22390016 Postdoctoral Fellowship, American Heart Association Lee (PI) 01/15 – 12/15
"Kir Channel Gating by Membrane Lipid Binding" **Award**: $48,428.00

1R03TR003670-01 NIH, NCATS Lee (PI) 04/21 – 08/22
"Structure/function analysis of understudied pH-sensitive Kir4.2 channels in vitro with conformation specific nanobodies" **Award**: $157,500

JIT1065 ICTS, Washington University Lee(PI) 10/23 – 12/24
"Kir4.1 Regulation by a Novel Auxiliary Protein" **Award**: $4,014