BIOGRAPHICAL SKETCH

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NAME: NIMIGEAN, CRINA M

eRA COMMONS USER NAME (credential, e.g., agency login): CNIMIGEAN

POSITION TITLE: 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)	END DATE MM/YYYY	FIELD OF STUDY
University of Bucharest, Bucharest	MS	06/1995	Physics
University of Bucharest, Bucharest	BS	06/1995	Physics
University of Miami School of Medicine	PHD	12/1999	Physiology and Biophysics
HHMI/Brandeis University	Postdoctoral Fellow	04/2003	Biochemistry
Brandeis University, Waltham, MA	NIH training grant	04/2005	Biochemistry

A. Personal Statement

Research in my laboratory is geared toward understanding how ion channel protein structure and mechanism interrelate at the molecular level to allow channels to elaborate various biological properties. The main focus of the lab is to elucidate the mechanisms of gating, ligand modulation, and lipid/membrane modulation in ion channels. We approach these questions using a range of biological and biophysical techniques, including electrophysiology, stopped-flow fluorescence assays and single-particle cryo-electron microscopy (cryo-EM). Our lab has extensive documented expertise in electrophysiology, using both voltage-clamp in cells and lipid bilayer recordings from purified ion channels in liposomes, stopped-flow fluorometry assays, which were shown to be particularly useful to study ensemble properties of purified ion channels in liposomes, as well as in solving structures of ion channels in detergents and lipid nanodiscs using single-particle cryo-EM. We have several long-time collaborations with MD simulations and AFM experts in ion channels such as Alessio Accardi and Simon Scheuring from Weill Cornell. Cryo-EM combined with high-resolution single-channel recordings, flux assays, dynamical measurements with AFM, and MD simulations can provide an unparalleled wealth of information into the molecular mechanism of ion channel gating and modulation. We made tremendous progress on understanding gating in potassium channels by deciphering the key states in the MthK channel gating mechanism, discovering the structural correlates of ball-and-chain inactivation, understanding the reason why the selectivity filter in MthK does not collapse to inactivate the channel as in KcsA, and identifying novel state dependent membrane-facing fenestrations that we showed allow entry of compounds into the pore from the bilayer instead of the canonical ion conduction pathway. In addition, we also made great progress in understanding how cyclic nucleotide-modulated channels gate at the molecular level and how they select between cyclic nucleotides, how anionic lipids increase their activity, how voltage increases their activity, and how enzymes modulate their activation kinetics.

1. Li C-C, Nimigean CM. Mechanism of lipid-dependent thermosensation in an ion channel, *bioRXiv,* June 5, 2025, doi: https://doi.org/10.1101/2025.06.03.657524.

2. Thon O, Wang Z, Schmidpeter PAM, Nimigean CM. PIP2 inhibits pore opening of the cyclic nucleotide-gated channel SthK. Nat Commun. 2024 Sep 19;15(1):8230. PubMed Central PMCID: PMC11413322.
3. Schmidpeter PAM, Wu D, Rheinberger J, Riegelhaupt PM, Tang H, Robinson CV, Nimigean CM. Anionic lipids unlock the gates of select ion channels in the pacemaker family. Nat Struct Mol Biol. 2022 Nov;29(11):1092-1100. PubMed Central PMCID: PMC10022520.
4. Fan C, Sukomon N, Flood E, Rheinberger J, Allen TW, Nimigean CM. Ball-and-chain inactivation in a calcium-gated potassium channel. Nature. 2020 Apr;580(7802):288-293. PubMed Central PMCID: PMC7153497.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2020 -	Professor, Weill Cornell Medical College, New York, NY
2011 - 2020	Associate Professor, WEILL MEDICAL COLL OF CORNELL UNIV
2008 - 2011	Assistant Professor, WEILL MEDICAL COLL OF CORNELL UNIV
2005 - 2008	Assistant Professor, UNIVERSITY OF CALIFORNIA AT DAVIS

Honors

2025	Kenneth S. Cole Award, Biophysical Society
2024	Acting co-chair, Gordon Research Conference: “Ligand Recognition and Molecular Gating” Ventura, CA
2022 - 2025	Permanent Study Section Member, BBM, NIH
2020 - 2026	Associate editor, Journal of General Physiology
2020 - 2022	President, Society of General Physiologists
2019 - 2020	Chair, "Channels, Receptors, and Transporters subgroup of the Biophysical Society
2016	Chair, Ligand Recognition and Molecular Gating GRC
2012	Biophysicist in profile, Biophysical Society Newsletter
2011 - 2020	Editorial board member, Journal of General Physiology
2011 - 2013	Councilor, Society of General Physiologists
2011 - 2012	Chair, Permeation and Transport subgroup of the Biophysical Society
2011	Career Scientist Award, Irma T. Hirschl Trust
2010	Most Promising Young Woman in Biophysics Margaret Dayhoff Award, Biophysical Society
2007 - 2022	Scientific Grant Reviewer, NSF, NIH, Wellcome Trust, American Heart Association, Italian Ministry of Health, Israeli Science Foundation, INSERM/CNRS Avenir, Fladers Research Foundation, Deutsche Forschungsgemeinschaft
2006	Scientist Development Award, American Heart Association - National
2000	Postdoctoral fellowship, Howard Hughes Medical Institute
1999	Academic excellence merit award, University of Miami Graduate School
1998	Predoctoral fellowship, American Heart Association - Florida affiliate
1995	National Scholarship Award, University of Bucharest, Romania
1995	Fellowship award, TEMPUS, University of Coimbra, Portugal

C. Contribution to Science

1. We contributed to the understanding of the fundamental mechanisms of pH gating and selectivity for K+ and against Na+ in K channels with single-channel recording, X-ray crystallography, and molecular dynamics simulations. Rejection of Na+ from K channels is crucial for excitability and understanding this process is of fundamental importance. Our findings challenged commonly held ideas about permeation and selectivity in K channels as we proposed that Na+ and Li+ are also favored to bind at the selectivity filter, albeit at different sites than K+, and that selectivity is due to a large entry barrier for Na+ and Li+. We gained insights into the mechanism by which different K channels with the same signature sequence (GYG) achieve different selectivities by taking advantage of a non-inactivating KcsA variant with less selectivity. We found that filters that do not collapse/inactivate are also less selective for K+. For pH gating in KcsA, we identified a histidine and a glutamate at the intracellular pore entrance, necessary to render KcsA pH dependent. The pH sensor residues are located within a lager network of ionizable residues at the bundle crossing and we dissected the individual contributions of these residues to pH sensing.

    a. Posson DJ, Thompson AN, McCoy JG, Nimigean CM. Molecular interactions involved in proton-dependent gating in KcsA potassium channels. J Gen Physiol. 2013 Dec;142(6):613-24. PubMed Central PMCID: PMC3840921.

b. Cheng WW, McCoy JG, Thompson AN, Nichols CG, Nimigean CM. Mechanism for selectivity-inactivation coupling in KcsA potassium channels. Proc Natl Acad Sci U S A. 2011 Mar 29;108(13):5272-7. PubMed Central PMCID: PMC3069191.

c. Thompson AN, Kim I, Panosian TD, Iverson TM, Allen TW, Nimigean CM. Mechanism of potassium-channel selectivity revealed by Na(+) and Li(+) binding sites within the KcsA pore. Nat Struct Mol Biol. 2009 Dec;16(12):1317-24. PubMed Central PMCID: PMC2825899.

d. Thompson AN, Posson DJ, Parsa PV, Nimigean CM. Molecular mechanism of pH sensing in KcsA potassium channels. Proc Natl Acad Sci U S A. 2008 May 13;105(19):6900-5. PubMed Central PMCID: PMC2383984.

2. We contributed towards deciphering the mechanism of gating in potassium channels using the prokaryotic MthK, a Ca2+-activated K channel, and KcsA, as models. Eukaryotic Ca2+-activated K (BK) channels have important physiological roles, and understanding their gating is fundamental. Using structural, biophysical, and electrophysiological techniques, we characterized the channel and identified the location of the gates that open and close MthK with voltage. We proposed that the activation gate is at the selectivity filter rather than at the "bundle-crossing", where voltage-gated ion channels and KcsA are believed to gate, in agreement with functional/structural data on BK channels. This finding opened the question of whether other channels may also gate at the selectivity filter. Using MD simulations and single-channel recordings of KcsA mutants, we found that KcsA also gates at the selectivity filter. We proposed a universal mechanism of activation in K channels where the central gate for ions is located at the selectivity filter and the movement of the inner helices couples ligand binding to the filter gate. The cryo-EM structures of Ca2+-free and Ca2+-bound (closed and open) MthK channels we determined were surprising from this perspective. The closed conformation displayed a tightly shut bundle-crossing, indicating it as one of the gates. Our investigations, showed that the blocker molecules that we and others used to probe state-dependent access to the pore of these channels, enter the closed state pore via lipid-facing fenestrations rather than via the ion conduction pathway. These fenestrations only exist in the closed state, are conserved in BK channels, and revived the concept of a selectivity filter gate.

a. Fan C, Flood E, Sukomon N, Agarwal S, Allen TW, Nimigean CM. Calcium-gated potassium channel blockade via membrane-facing fenestrations. Nat Chem Biol. 2024 Jan;20(1):52-61. PubMed Central PMCID: PMC10847966.

b. Heer FT, Posson DJ, Wojtas-Niziurski W, Nimigean CM, Bernèche S. Mechanism of activation at the selectivity filter of the KcsA K(+) channel. Elife. 2017 Oct 10;6 PubMed Central PMCID: PMC5669632.

c. Posson DJ, Rusinova R, Andersen OS, Nimigean CM. Calcium ions open a selectivity filter gate during activation of the MthK potassium channel. Nat Commun. 2015 Sep 23;6:8342. PubMed Central PMCID: PMC4580985.

d. Posson DJ, McCoy JG, Nimigean CM. The voltage-dependent gate in MthK potassium channels is located at the selectivity filter. Nat Struct Mol Biol. 2013 Feb;20(2):159-66. PubMed Central PMCID: PMC3565016.

3. We reported the first structural correlates of “ball-and-chain” inactivation in a K channel. Inactivation is a universal process by which ion channels terminate ion flux through their pores while opening stimulus is still present. In neurons, channel inactivation is crucial for action potential generation and firing frequency regulation. N-type and C-type inactivation are two major inactivation processes in K channels. N-type inactivation was proposed to involve a cytoplasmic domain plugging the open pore via a “ball-and-chain” mechanism. C-type inactivation was proposed to involve a selectivity filter modification (controversially, some found a constriction and others a dilation) to stop permeation. Although "ball-and-chain" inactivation was coined as early as 1973, structural evidence was first provided by us in 2020 when we determined structures of a Ca2+-gated and inactivating channel (MthK) in a lipid environment using single-particle cryo-EM. The open channel conformations revealed that the N-terminus of one subunit of the tetramer sticks into the pore and plugs it. Deletion of N-terminus leads to non-inactivating channels indicating that this N-terminal peptide is responsible for ball-and-chain inactivation. In a recent paper, we report a similar “ball-and-chain” mechanism for human Ca2+-gated K channels inactivated by accessory beta subunits. MthK channels also proved a good model for insights toward C-type inactivation. We found that MthK, despite having the same selectivity filter structure and sequence as C-type inactivating KcsA channels, do not undergo C-type inactivation because the selectivity filter has a higher binding affinity for permeant ions,

due to molecular interactions occurring behind the filter. Ca2+-induced MthK conformational changes were also used to validate Spotiton as a time-resolved cryo-EM technology.

a. Agarwal S, Kim ED, Lee S, Simon A, Accardi A, Nimigean CM. Ball-and-chain inactivation of a human large conductance calcium-activated potassium channel. Nat Commun. 2025 Feb 19;16(1):1769. PubMed Central PMCID: PMC11840039.
b. Boiteux C, Posson DJ, Allen TW, Nimigean CM. Selectivity filter ion binding affinity determines inactivation in a potassium channel. Proc Natl Acad Sci U S A. 2020 Nov 24;117(47):29968-29978. PubMed Central PMCID: PMC7703589.
c. Dandey VP, Budell WC, Wei H, Bobe D, Maruthi K, Kopylov M, Eng ET, Kahn PA, Hinshaw JE, Kundu N, Nimigean CM, Fan C, Sukomon N, Darst SA, Saecker RM, Chen J, Malone B, Potter CS, Carragher B. Time-resolved cryo-EM using Spotiton. Nat Methods. 2020 Sep;17(9):897-900. PubMed Central PMCID: PMC7799389.
d. Fan C, Sukomon N, Flood E, Rheinberger J, Allen TW, Nimigean CM. Ball-and-chain inactivation in a calcium-gated potassium channel. Nature. 2020 Apr;580(7802):288-293. PubMed Central PMCID: PMC7153497.

4. We identified and functionally characterized two prokaryotic cyclic nucleotide-modulated channels (MloK1 and SthK), good structural and biochemical models for eukaryotic cyclic nucleotide-modulated (CNG/HCN) channels. These channels are central to visual and olfactory signal transduction, as well as the pacemaker activity in the heart and brain. By relating the function of these channels to their structure, it will ultimately be possible to develop/identify pharmaceutical agents that could either enhance or block channel activity, depending on the target and the ultimate goal. The original model, MloK1, a CNG channel from M. loti, was identified and characterized by us starting in 2004, and in collaboration with other investigators, we determined a series of MloK1 structures using single particle and cryo-electron crystallography, and we visualized conformational changes upon ligand binding with high-speed AFM (HS-AFM). In 2018, we characterized a superior structural and functional model for CNG channels: SthK from S. thermophila. SthK is highly homologous with eukaryotic CNG and HCN channels and is functional in both ensemble assays and single-channel lipid bilayer recordings. Using cryo-EM, we solved high-resolution SthK structures in different ligand-bound conformations. Because its high expression makes it amenable to biophysical, biochemical, functional and structural assays, SthK investigations led to many breakthroughs in our understanding of these channels. We elucidated the conformational changes associated with channel gating in response to cyclic nucleotide binding and to membrane depolarization, using single-particle cryo-EM, HS-AFM, single-molecule FRET, electrophysiology, and stopped-flow assays. We identified a series of on-path conformational intermediates during channel activation revealing an inhibitory role for the voltage sensor domain at rest as well as a non-canonical motion undergone by the voltage sensor in response to depolarization. We identified a completely novel means of channel modulation via an enzyme, prolyl isomerase, that is normally involved in protein folding. This mechanism would allow cellular enzymes to fine tune the activity of the channels, which could play an important role in the regulation of pacemaker channels in vivo, and allow for heart rhythm tuning. As described in the next section, SthK has also been instrumental in our initial investigations regarding lipid modulation.

a. Gao X, Schmidpeter PAM, Berka V, Durham RJ, Fan C, Jayaraman V, Nimigean CM. Gating intermediates reveal inhibitory role of the voltage sensor in a cyclic nucleotide-modulated ion channel. Nat Commun. 2022 Nov 14;13(1):6919. PubMed Central PMCID: PMC9663499.
b. Schmidpeter PAM, Rheinberger J, Nimigean CM. Prolyl isomerization controls activation kinetics of a cyclic nucleotide-gated ion channel. Nat Commun. 2020 Dec 16;11(1):6401. PubMed Central PMCID: PMC7744796.
c. Marchesi A, Gao X, Adaixo R, Rheinberger J, Stahlberg H, Nimigean C, Scheuring S. An iris diaphragm mechanism to gate a cyclic nucleotide-gated ion channel. Nat Commun. 2018 Sep 28;9(1):3978. PubMed Central PMCID: PMC6162275.
d. Rheinberger J, Gao X, Schmidpeter PA, Nimigean CM. Ligand discrimination and gating in cyclic nucleotide-gated ion channels from apo and partial agonist-bound cryo-EM structures. Elife. 2018 Jul 20;7 PubMed Central PMCID: PMC6093708.

5. Recently we have been exploring the druggability and the mechanism of lipid modulation of ion channels in the family of cyclic nucleotide-gated ion channels. We determined that the small molecule propofol allosterically inhibits human HCN1 (hyperpolarization-activated and cyclic nucleotide-gated) channel activity by binding at a novel mechanistic hotspot where it enhances the coupling between the voltage sensor and the channel gate. In the process, we discovered that propofol application can restore normal voltage-dependent gating to two leaky HCN1 channel mutants associated with epilepsy, and we are currently following this potential therapeutic avenue. In addition, we have commenced investigations into the mechanism of lipid modulation of cyclic nucleotide-gated channels. Little quantitative information exists about how membrane-forming or signaling lipids modulate ion channels in general, mainly due to the difficulty of controlling the lipid composition and concentration since ion channel activity had been previously investigated mainly in cells, where lipid manipulation is limited. By employing purified ion channels in liposomes and lipid nanodiscs and performing high resolution native mass spectrometry, structural, and functional methods, we determined that anionic lipids (such as PA, PG) increase the activity of prokaryotic SthK channels through weakening a conserved salt-bridge that normally stabilizes the closed state. Qualitative enzymatic manipulation of cellular lipids indicated that eukaryotic HCN2 channels likely use a similar mechanism to increase their activity in response to PA lipids. Using similar quantitative approaches, we also recently reported the mechanism by which PIP2 lipids inhibit gating of SthK channels and are currently investigating how lipids modulate eukaryotic CNG and HCN channels.

    a. Thon O, Wang Z, Schmidpeter PAM, Nimigean CM. PIP2 inhibits pore opening of the cyclic nucleotide-gated channel SthK. Nat Commun. 2024 Sep 19;15(1):8230. PubMed Central PMCID: PMC11413322.
    b. Kim ED, Wu X, Lee S, Tibbs GR, Cunningham KP, Di Zanni E, Perez ME, Goldstein PA, Accardi A, Larsson HP, Nimigean CM. Propofol rescues voltage-dependent gating of HCN1 channel epilepsy mutants. Nature. 2024 Aug;632(8024):451-459. PubMed Central PMCID: PMC11634041.
    c. Schmidpeter PAM, Petroff JT 2nd, Khajoueinejad L, Wague A, Frankfater C, Cheng WWL, Nimigean CM, Riegelhaupt PM. Membrane phospholipids control gating of the mechanosensitive potassium leak channel TREK1. Nat Commun. 2023 Feb 25;14(1):1077. PubMed Central PMCID: PMC9968290.
    d. Schmidpeter PAM, Wu D, Rheinberger J, Riegelhaupt PM, Tang H, Robinson CV, Nimigean CM. Anionic lipids unlock the gates of select ion channels in the pacemaker family. Nat Struct Mol Biol. 2022 Nov;29(11):1092-1100. PubMed Central PMCID: PMC10022520.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/crina.nimigean.1/bibliography/public/

BIOGRAPHICAL SKETCH

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NAME: Chenglong Jin

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Postdoctoral Associate

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
China Pharmaceutical University, Nanjing, China	B.S.	06/2015	Pharmaceutical Engineering
Seoul National University, Seoul, Republic of Korea	M.S.	08/2017	Pharmacy
Seoul National University, Seoul, Republic of Korea	Ph.D.	08/2021	Pharmacy
Seoul National University, Seoul, Republic of Korea	Postdoc	08/2024	Pharmacy
Weill Cornell Medical College, New York, NY, USA	Postdoc	Present	Anesthesiology

A. Personal Statement

I am a Postdoctoral Associate in the Department of Anesthesiology at Weill Cornell Medicine. My current research focuses on elucidating the molecular mechanisms of inactivation in human large-conductance calcium-activated potassium (BK) channels, using a multidisciplinary approach that combines molecular biology, biochemistry, electrophysiology, and cryo-electron microscopy (cryo-EM). I received my Ph.D. under the mentorship of Prof. Bong-Jin Lee at Seoul National University, where I also continued as a postdoctoral researcher. During that time, my work centered on the structural and functional characterization of toxin-antitoxin (TA) systems in pathogenic bacteria. I determined several crystal structures of type II and type VII TA complexes, providing significant insights into the dynamic interactions between RNase-acting toxins and their cognate antitoxins, which regulate toxin activity either through direct inhibition or allosteric mechanisms. I also expanded my research to identify peptides and small molecules that disrupt these complexes and activate toxins.

Since November 2024, I have joined Prof. Crina Nimigean's lab at Weill Cornell Medicine to broaden my expertise in ion channel research. My current project investigates the molecular basis of β2 subunit-mediated inactivation in BK channels. BK channels exhibit exceptionally high conductance (~300 pS) and are key mediators of membrane hyperpolarization. They are activated by two physiological stimuli: intracellular Ca^{2+} and membrane depolarization. The functional properties of BK channels—including voltage sensitivity, calcium dependence, and pharmacological profile—are modulated by auxiliary β and γ subunits. Among them, the β2 subunit, uniquely induces rapid and complete channel inactivation. Inactivation refers to the cessation of ion flow despite the continued presence of activating stimuli and plays an essential role in regulating neuronal excitability, such as use-dependent spike broadening observed in hypothalamic and amygdalar neurons. Previous structural studies have resolved BK channels alone or in complex with auxiliary subunits in detergents and nanodiscs, including a chimeric construct in which the β2 N-terminus was grafted onto the non-inactivating β4 scaffold. These studies identified three key hydrophobic residues in the β2 N-terminus as crucial for inactivation via pore occlusion. However, structural data for BK channels bound to full-length β2 subunits—especially in native-like lipid

environments—remain unavailable. Determining such structures, particularly in proteoliposomes, would enable mechanistic insights into β2-mediated inactivation under more physiologically relevant conditions.

Given the complexity of this system, I believe the advanced instrumentation and technical expertise available at NCCAT are essential for achieving high-resolution structural determination. This project aligns closely with NCCAT's mission to support transformative research on challenging membrane protein systems.

B. Positions, Scientific Appointments, and Honors

2024 – present	Postdoctoral Associate, Weill Cornell Medical College, New York, NY, USA
2021 – 2024	Postdoctoral Associate, Seoul National University, Seoul, Republic of Korea

Honors

2021	Best paper award, Biolipids Interactomics Research Center, College of Pharmacy, Seoul National University, Republic of Korea.
2020	Best paper award, Brain Korea 21, College of Pharmacy, Seoul National University, Republic of Korea.
09/2015-08/2019	OK Fellowship, Overseas Koreans Foundation, Republic of Korea
09/2015-08/2018	SNU Global Scholarship, Seoul National University, Republic of Korea
11/2016	Wooduk-foundation Scholarship, Wooduk foundation, Republic of Korea

C. Contributions to Science

1. Structural and functional study of Toxin-Antitoxin (TA) system in pathogenic bacteria.

My previous research focused on the structural and functional characterization of bacterial toxin–antitoxin (TA) systems. Specifically, I resolved multiple crystal structures of TA complexes from both type II and type VII modules, revealing the molecular basis of the dynamic interplay between RNase-acting protein toxins and their cognate antitoxins, which neutralize toxicity through direct binding or allosteric inhibition. In parallel, I investigated peptides and small molecules capable of disrupting these complexes to trigger toxin activation. These studies have advanced our mechanistic understanding of TA systems and hold promise for the development of novel antimicrobial strategies targeting persistent bacterial populations.

a. Do-Hee Kim, Yong-Chan Lee, **Chenglong Jin**, Sung-Min Kang, Su-Jin Kang, Hoon-Seok Kang & Bong-Jin Lee, Structural and functional insight into YefM-YoeB complex of Toxin-Antitoxin system from *Streptococcus pneumoniae*, *Journal of Cellular Biochemistry* (2025), Jan;126(1):e30672.

b. **Chenglong Jin*****, Cha-Hee Jeon*****, Heung Wan Kim, Jin Mo Kang, Yuri Choi, Sung-Min Kang, Hyung Ho Lee, Do-Hee Kim, Byung Woo Han & Bong-Jin Lee, Structural insight into the distinct regulatory mechanism of the HEPN–MNT toxin-antitoxin system in *Legionella pneumophila*, *Nature Communications* (2024), Nov 24; 15,10188

c. **Chenglong Jin***, Sung-Min Kang*, Do-Hee Kim*, Yuno Lee & Bong-Jin Lee, Discovery of antimicrobial agents based on structural and functional study of the *Klebsiella pneumoniae* MazEF Toxin–Antitoxin System, *Antibiotics* (2024), Apr 26; 13(5), 398

d. Do-Hee Kim, Youngseo Na, Heesun Chang, Jun-Hyuk Boo, Sung-Min Kang, **Chenglong Jin,** Su-Jin Kang, Su Yeon Lee & Bong-Jin Lee, Domain swapping of the C-terminal helix promotes the

dimerization of a novel ribonuclease protein from *Mycobacterium tuberculosis*. *Protein Science* (2023), Jun; 32(6): e4644

e. **Chenglong Jin***, Sung-Min Kang*, Do-Hee Kim* & Bong-Jin Lee, Structural and functional analysis of the *Klebsiella pneumoniae* MazEF toxin-antitoxin system. *IUCrJ* (2021), Mar 5; 8(Pt 3): 362-371

f. Sung-Min Kang*, **Chenglong Jin***, Do-Hee Kim*, Sung Jean Park, Sang-Woo Han & Bong-Jin Lee, Structure-based design of peptides that trigger *Streptococcus pneumoniae* cell death. *FEBS JOURNAL* (2021), Mar; 288(5), 1546–1564

g. Sung-Min Kang*, **Chenglong Jin***, Do-Hee Kim*, Yuno Lee & Bong-Jin Lee, Structural and functional study of the *Klebsiella pneumoniae* VapBC toxin-antitoxin system, including the development of an inhibitor that activates VapC. *Journal of Medicinal Chemistry* (2020), Nov 25; 63(22), 13669-13679

h. Sung-Min Kang, Do-Hee Kim, **Chenglong Jin**, Hee-Chul Ahn & Bong-Jin Lee, The crystal structure of AcrR from *Mycobacterium tuberculosis* reveals a one-component transcriptional regulation mechanism. *FEBS OPEN BIO* (2019), Oct; 9(10), 1713~1725

i. Sung-Min Kang, Do-Hee Kim, **Chenglong Jin** & Bong-Jin Lee, A systematic overview of type II and III toxin-antitoxin systems with a focus on druggability. *TOXINS* (Basel) (2018), Dec 4; 10(12): 515

j. Do-Hee Kim, Sung-Min Kang, Sung Jean Park, **Chenglong Jin**, Hye-Jin Yoon & Bong-Jin Lee, Functional insights into the *Streptococcus pneumoniae* HicBA toxin-antitoxin system based on a structural study. *Nucleic Acids Research* (2018), Jul 6; 46(12): 6371-6386

*Co-first author

Patents

a. Bong-Jin Lee & **Chenglong Jin**, Antibacterial peptides and compounds targeting toxin-antitoxin system of *Klebsiella pneumoniae*, and their use; South Korea Patent, #1025815000000, Sep 18, 2023
b. Bong-Jin Lee & **Chenglong Jin**, Antimicrobial peptides increasing toxicities of endogenous toxin and targeting HigBA toxin-antitoxin system of *Streptococcus pneumoniae*, and their use; South Korea Patent, #1025510380000, Jun 29, 2023

2. Current research on calcium-activated potassium channels

a. **Inactivation mechanism of β2 subunit to BK channels in vesicles.** BK channels are large-conductance (~300 pS), calcium- and voltage-activated potassium channels that open in response to both cytosolic Ca^{2+} elevation and membrane depolarization. In mammals, the pore-forming BK α-subunit associates with auxiliary β and γ subunits that modulate gating properties in a tissue-specific manner, influencing voltage sensitivity, calcium dependence, and pharmacological profiles. Among these, the β2 subunit—expressed in the brain, pancreas, and adrenal chromaffin cells—confers rapid and complete inactivation of BK channels. Inactivation refers to the cessation of ion flux despite the continued presence of activating stimuli and serves critical roles in shaping neuronal excitability. For example, β2-mediated inactivation has been implicated in use-dependent spike broadening in hypothalamic pyramidal neurons and lateral amygdala neurons, potentially enhancing synaptic transmission. Elucidating the structure of BK channels in complex with full-length β2, particularly in proteoliposomes that mimic native membrane environments, is essential to uncover the mechanistic basis of β2-mediated inactivation under physiologically relevant conditions.

b. **<u>Cholesterol-mediated modulation of BK channels in asymmetric lipid environments</u>** In eukaryotic cells, the lipid composition of the plasma and organellar membranes is asymmetrically distributed between the two leaflets of the bilayer. Phosphatidylcholine (PC) and sphingomyelin are predominantly localized to the outer leaflet, whereas phosphatidylserine (PS) and phosphatidylethanolamine (PE) are enriched in the inner leaflet. This lipid asymmetry is dynamically maintained and plays critical roles in membrane biogenesis, vesicular trafficking, cell signaling, and apoptosis. Cholesterol, which is reported to be enriched in the outer leaflet, has been implicated in the inhibition of BK channel activity, potentially through direct cholesterol–BK channel interactions. However, the precise molecular mechanisms underlying this inhibitory effect remain poorly understood. To investigate this, we employed lipid transporters such as flippases and floppases to induce leaflet-specific cholesterol asymmetry in synthetic liposomes. We then reconstituted BK channels into these cholesterol-asymmetric liposomes to explore the functional consequences and structural basis of cholesterol-mediated modulation. Given the pathophysiological relevance of both BK channel function and cholesterol homeostasis, elucidating the molecular details of their interaction under asymmetric membrane conditions may provide novel insights into membrane protein regulation and offer potential avenues for therapeutic targeting.

Complete List of Published Work in MyBibliography:

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BIOGRAPHICAL SKETCH

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NAME: Taehyun Park

eRA COMMONS USER NAME (credential, e.g., agency login): PARK.TAEHYUN

POSITION TITLE: Postdoctoral Associate

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
Hanyang University, Seoul, Republic of Korea	B.S.	02/2013	Bioengineering
Hanyang University, Seoul, Republic of Korea	Ph.D.	08/2021	Bioengineering
National Cancer Center Korea, Republic of Korea	Postdoc	12/2021	Precision Medicine
Weill Cornell Medical College	Postdoc	Present	Anesthesiology

A. Personal Statement

I am a structural biologist broadly interested in how small molecules, lipids, and protein partners modulate membrane protein function. My early training at the National Cancer Center Korea focused on structure-based drug discovery targeting proteins overexpressed in cancer. I later conducted structural and functional studies on PTPIP51, a mitochondrial lipid transport protein, which sparked my long-term interest in lipid-mediated regulation of membrane proteins. Since 2022, I have been working in the Crina Nimigean Lab at Weill Cornell Medicine to investigate the gating mechanisms of cyclic nucleotide-gated (CNG) channels in sensory systems.

My current projects focus on the rod and olfactory CNG channels, which play essential roles in visual and olfactory signal transduction, respectively.

In the projects, 1) I aim to determine how phospholipids (PE, PS) and PIP2 regulate rod CNG channel activity. I have already determined the structure of the CNGA1 subunit reconstituted in lipid nanodiscs, and successfully expressed the full heterotetrameric rod CNG channel (CNGA1/CNGB1). Using functional assays and single-particle cryo-EM, I have elucidated the structural mechanism by which PIP2 inhibits the rod CNG channel—stabilizing a closed conformation and reducing its cGMP sensitivity. In contrast, although PE and PS enhance channel activity by increasing cGMP sensitivity, the structural basis for this lipid-mediated activation remains unknown. Additionally, while a recent cryo-EM study has resolved the structure of the rod CNG channel in detergent, the fully open conformation of the heterotetrameric rod CNG channel remains to be captured. This aim seeks to reveal the structural mechanisms underlying lipid-mediated modulation of the rod CNG channel.

2) I aim to determine the gating mechanism of the olfactory CNG channel. I have already solved cryo-EM structures of the olfactory CNG channel (CNGA2/CNGA4/CNGB1) in detergent, capturing both closed and partially open conformations. Further study will focus on obtaining high-resolution structures of the olfactory channel in lipid nanodiscs and elucidating the effects of ligands on its gating dynamics.

To address this gap, I have confirmed successful expression of the heterotetrameric rod CNG channel and am reconstituting the purified protein into lipid nanodiscs. Similarly, I have successfully expressed and purified the olfactory CNG channel, which I am also incorporating into lipid nanodiscs. I am employing single-particle cryo-EM to determine their structures in multiple functional states.

NCCAT's cutting-edge instrumentation and technical support are essential for this work. Given the biochemical complexity of these channels and the need to resolve subtle conformational transitions, repeated access to high-end cryo-EM platforms is critical. I have extensive experience in nanodisc reconstitution, grid preparation, and

cryo-EM data acquisition and processing. With a clearly defined scientific objective and demonstrated expertise, I believe this project is well aligned with NCCAT's mission and will benefit greatly from its support.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2022 – present	Postdoctoral Associate, Weill Cornell Medical College
2021 – 2021	Postdoctoral Associate, National Cancer Center Korea, Republic of Korea
2018 – 2021	Technical Research Personnel (alternative military service), National Cancer Center Korea, Republic of Korea

After my Ph.D. coursework, I started alternative military service as technical research personnel in National Cancer center, Korea. I did research for three years rather than serving as a soldier, which is categorized as an alternative military service for only Korean male people who finished Ph.D. coursework or master's degree. My task in National Cancer Center was to identify cancer-targeting drug candidates based on structural biological insight under the supervision of Dr. Byung Il Lee. After my Ph.D. dissertation, as a postdoctoral associate, I continued the projects I had studied on in the same lab of National Cancer Center.
In February 2022, I joined the Crina Nimigean Lab, Weill Medical College of Cornell University, USA. And I've been working on the projects about the molecular mechanism of human Cyclic Nucleotide-Gated (CNG) channels for 3 years

Other Professional Memberships

2025 – present	Member, the Society of General Physiologists
2024 – present	Member, Biophysical Society
2023 – present	Member, The New York Academy of Sciences
2022 – present	Member, New York Korean Scientists

Honors

2021	Best Research Fellow Award 2021, January 3rd, 2022, *National Cancer Center*, Korea.
2021	Young Scientist Award*, the 15th Annual Conference of Korean Society for Mitochondria Research and Medicine (KSMRM)*, August 25th, 2021, held in BEXCO, Busan, Korea, "Phospholipid-transfer function of PTPIP51 at mitochondria-associated ER membranes"
2021	Poster Award, *Korean Society for Protein Science & The Korean Biophysical Society Joint Symposium*, October 22nd, 2021, held in KRIBB, Daejeon, Korea, "Phospholipid-transfer function of PTPIP51 at mitochondria-associated ER membranes"

C. Contributions to Science

1. **Graduate career**
    a. **Identifying the anti-tumor drug candidates based on structural and functional analysis of cell signaling modulators.** Cell signaling plays a critical role in regulating cellular functions and coordinating multicellular processes such as tissue repair, development, and immunity. Dysregulation or overexpression of signaling components can lead to diseases, particularly cancers, emphasizing the importance of understanding these pathways. To address this, I focused on identifying tumor-targeting drug candidates by inhibiting key targets involved in signaling dysregulation, including kinase/phosphatase enzymes and scaffold proteins such as DUSP1/6, MERTK, Axl, ROCK1, ROCK2, and BRD4.
        i. **Tae Hyun Park***, Jung-Hoon Kim*, Navin Pandit*, Miyoun Yoo*, Ji U Choi, Chi Hoon Park, Kwan-Young Jung, Byung Il Lee, Crystal structure of [1, 2, 4] triazolo [4, 3-b] pyridazine derivatives as BRD4 bromodomain inhibitors and structure–activity relationship study, *Scientific Reports (2023)* PMCID: PMC10319850
        ii. **Tae Hyun Park***, Navin Pandit*, Miyoun Yoo*, Jiin Kim, Seul Mi Kim, Kyu Myung Lee, Yeongrin Kim, Seoung Min Bong, Byung Il Lee, Kwan-Young Jung, Chi Hoon Park, Discovery of BET specific bromodomain inhibitors with a novel scaffold, *Bioorganic & Medicinal Chemistry (2022)*

iii. Seung-Hyun Bae, Jung-Hoon Kim, **Tae Hyun Park**, Kyeong Lee, Byung Il Lee, Hyonchol Jang, BMS794833 inhibits macrophage efferocytosis by directly binding to MERTK and inhibiting its activity, *Experimental & Molecular Medicine (2022)* PMCID: PMC9534909

iv. **Tae Hyun Park***, Minjin Yoo*, Miyoun Yoo*, Yeongrin Kim, Joo-Youn Lee, Kyu Myung Lee, Seong Eon Ryu, Byung Il Lee, Kwan-Young Jung, Chi Hoon Park, Synthesis and Structure–Activity Relationships of Aristoyagonine Derivatives as Brd4 Bromodomain Inhibitors with X-ray Co- Crystal Research, *Molecules (2021)* PMCID: PMC8002823

v. **Tae Hyun Park***, Seung-Hyun Bae, Seoung Min Bong, Seong Eon Ryu, Hyonchol Jang, Byung Il Lee, Crystal Structure of the Kinase Domain of MerTK in Complex with AZD7762 Provides Clues for Structure-Based Drug Development*, International Journal of Molecular Sciences (2020)* PMCID: PMC7660649
*Co-first author

Patents

1. RYU, Seong Eon, **PARK, Tae Hyun**, LEE, Kwang Hwan, KANG, Ju Seop, KIM, Shin Hee, NAM, Kyoung Tae, LEE, Hyeon Kyu, "Pharmaceutical Composition Containing DUSP1 Inhibitor", KR- Registration No. 10-1882790-0000, US- Registration No. 11147807 and EU-Application No. 18783771.1.
2. LEE, Byung Il, JANG, Hyonchol, **PARK, Tae Hyun**, BAE, Seung-Hyun, "Composition for preventing, improving or treating cancer comprising TAM family kinase inhibitors ", KR-Application No. 10-2019-0097534
3. LEE, Byung Il, KIM, Kyung Tae, LEE, Kyeong, HARMALKAR, Dipesh, KIM, Min Kyoung, Lee, Hwa Young, PARK, Min Ji, **PARK, Tae Hyun**, BONG, Seung Min, LEE, Seung Jin, "Composition for preventing, improving or treating cancer comprising inhibitor of PLK1", KR-Registration No. 10- 2019-0097656

b. **Determining the first 3D crystal structure of PTPIP51, one of the main tethers of Mitochondria-EM interoranelle contact site and proving the activity of lipid transportation of PTPIP51.** Eukaryotic cells coordinate biochemical pathways across organelles through interorganelle communication. Mitochondria-associated ER membranes (MAMs) are key sites mediating processes like ER stress, mitochondrial dynamics, and apoptosis via MAM-tethering protein complexes. I studied PTPIP51. a mitochondrial membrane-anchored protein interacting with ER-bound VAPB. I solved the crystal structure of PTPIP51-TPR domain, and the structural analysis revealed the lipid binding and transfer ability of PTPIP51, specifically phosphatic acid (PA), and I demonstrated the PA transfer of PTPIP51 to mitochondria outer membrane can modulate the mitochondrial cardiolipin level.

i. **Tae Hyun Park***, Hyun Ku Yeo*, Hee Yeon Kim, Hyonchol Jang, Jueun Lee, Geum-Sook Hwang, Seong Eon Ryu, Si Hoon Park, Hyun Kyu Song, Hyun Seung Ban, Hye-Jin Yoon, Byung Il Lee, Phospholipid-transfer function of PTPIP51 at mitochondria-associated ER membranes, *EMBO reports (2021)* PMCID: PMC8183395
*Co-first author

2. **Postdoctoral Career**

a. **Identifying the mechanism of lipid modulation of human rod Cyclic Nucleotide-Gated channel.** The rod Cyclic Nucleotide-Gated (CNG) channel is an ion channel activated by cGMP, primarily expressed in rod photoreceptor cells, where it plays a critical role in phototransduction. Upon light activation of the rhodopsin cascade, intracellular cGMP level decreases, causing the rod CNG channel to close. This closure reduces calcium ion influx and alters the membrane potential, initiating the signal transmission necessary for light perception. Interestingly, PIP2 has been reported to inhibit the activity of the rod CNG channel by reducing its sensitivity to cGMP, though the molecular mechanism underlying this effect remains unclear. Through functional assays, I discovered that phospholipids such as PE and PS, which form cell membranes, also modulate the cGMP sensitivity of this channel. This regulation of cGMP sensitivity is highly significant, as the rod CNG channel translates rhodopsin signals into neural signals, directly influencing the eye's sensitivity to photons. To elucidate the molecular mechanisms of these lipid effects, I am employing advanced techniques such as stop-flow assays, single-channel

recordings, and lipid nanodisc-supported single-particle cryo-EM. My work aims to provide deeper insights into how these lipids regulate the rod CNG channel's function and its role in visual sensitivity.

i. **Taehyun Park**, Crina M Nimigean. The mechanism of PIP2 inhibitory activity of rod cyclic nucleotide-gated channel. *In preparation.*
ii. **Taehyun Park**, Crina M Nimigean. The modulation mechanisms of membrane-forming lipids of rod cyclic nucleotide-gated channel. *In preparation.*

b. **Identifying the gating mechanism of olfactory human rod Cyclic Nucleotide-Gated channel.** The olfactory CNG channel, primarily expressed in olfactory sensory neurons, is essential for odor detection and signal transduction. Activated by cyclic nucleotides such as cAMP and cGMP, it allows ion flux that generates neuronal responses required for the sense of smell. The channel is a heterotetramer composed of CNGA2, CNGA4, and CNGB1 subunits, and exhibits distinct gating behaviors compared to its rod counterpart. Despite its importance, the molecular mechanism underlying the gating of the human olfactory CNG channel remains unknown, and no high-resolution structural information is currently available. To address this gap, I have successfully expressed and purified the human olfactory CNG channel and am working to determine its structure using single-particle cryo-EM in lipid nanodiscs. This structural approach, combined with functional assays, will allow me to elucidate the conformational changes associated with channel opening and modulation.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/taehyun.park.1/bibliography/public/

BIOGRAPHICAL SKETCH

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NAME: Li, Chieh-Chin

eRA COMMONS USER NAME (credential, e.g., agency login): CHIEHCHIN.LI

POSITION TITLE: Postdoctoral Associate

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)	END DATE MM/YYYY	FIELD OF STUDY
National Tsing Hua University, Hsinchu	BS	06/2014	Chemistry
National Tsing Hua University, Hsinchu	PHD	03/2020	Chemistry
National Tsing Hua University, Hsinchu	Postdoctoral Fellow	12/2021	Chemistry
Weill Cornell Medicine, NEW YORK, New York	Postdoctoral Fellow	present	Anesthesiology

A. Personal Statement

My long-term research interest is in understanding the molecular mechanisms of thermosensation. During my Ph.D. at National Tsing Hua University, I studied the bacterial membrane protein BsYetJ, a homolog of the apoptotic protein TMBIM6, using electron spin resonance (ESR). This work sparked my interest in how membrane proteins respond to environmental stimuli, particularly temperature. Building on this interest, I joined Dr. Crina Nimigean's lab at Weill Cornell Medicine to investigate thermosensitive ion channels. My current research focuses on SthK from *Spirochaeta thermophila*, a thermophilic bacterial homolog of cyclic nucleotide-gated channels, and has identified two key features: (1) lipid-dependent temperature sensitivity and (2) a potential intersubunit salt bridge at the water-lipid interface that may act as a temperature sensor.

In this BAG application, I propose to investigate whether similar mechanisms apply to the eukaryotic cold thermoreceptor TRPM8, which shares several features with SthK and may employ a comparable mode of temperature sensing. Specifically, (1) both channels are activated by low temperatures; (2) both contain state-dependent salt bridges located at the water–lipid interface; and (3) these salt bridges are known to be sensitive to the surrounding lipid environment. Despite TRPM8's central role in cold sensation, the molecular basis of its activation by temperature remains unknown. While the open-channel cryo-EM structure of TRPM8 bound to a chemical agonist was resolved in 2022, a structure representing cold-induced activation is still unavailable. Furthermore, evolutionary analyses suggest that TRPM8's responses to cold and to chemical agonists are mechanistically distinct. This project aims to dissect the mechanism of temperature sensing in TRPM8, with a focus on lipid interactions and structural elements—such as salt bridges—that may mediate its cold activation.

Access to NCCAT's advanced cryo-EM resources and expert technical support will be instrumental for the success of this project. Given the conformational complexity of TRPM8 and the need to capture distinct functional states under varying thermal and lipid conditions, repeated use of high-resolution cryo-EM instrumentation is essential. I have substantial experience in reconstituting membrane proteins into nanodiscs, preparing cryo-EM grids, and performing data collection and analysis. With a focused research question and a strong background in membrane protein structural biology, this project is well positioned to leverage NCCAT's capabilities and contribute meaningfully to its scientific mission.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2022 - Postdoctoral Associate, Weill Cornell Medicine, New York, NY
2021 - 2021 Postdoctoral Associate, National Tsing Hua University, Hsinchu
2020 - 2021 Conscription Soldier, National Conscription Agency Ministry of the Interior

Honors

2022 - 2023	Postdoctoral Research Abroad Program Fellow, National Science and Technology Council, Taiwan
2020	Taiwan Rotary Academic Scholarship, The Chung Hwa Rotary Educational Foundation, Taiwan
2017	Excellent Poster Award, Taiwan Magnetic Resonance Society, Taiwan
2016	Best Oral Presentation Award, Kyoto University, Japan
2015	Travel Stipend Award, The European Federation of EPR groups, Germany
2014	NTHU Presidential Scholarship for Outstanding PhD Students with Research Potential, National Tsing Hua University, Taiwan

C. Contribution to Science

1. **Structure and Regulation of a Bacterial Homolog of an Apoptotic ER Membrane Protein**. The endoplasmic reticulum (ER) serves as the main intracellular reservoir of calcium, with its release regulating various cellular functions and playing a critical role in apoptosis. The transmembrane BAX inhibitor-1 motif-containing protein 6 (TMBIM6) is an evolutionarily conserved, multifunctional ER membrane protein that maintains calcium homeostasis, providing protection against apoptosis and ER stress. Using electron spin resonance (ESR) spectroscopy, I investigated a bacterial homolog of TMBIM6, BsYetJ, embedded in lipid nanodiscs and identified several previously unrecognized conformational substates involved in the calcium transport process. These findings support a model for calcium-release channels, indicating that membrane protein function requires transitions between distinct conformations within a preexisting conformational equilibrium.

    a. **Li CC**, Kao TY, Cheng CC, Chiang YW. Structure and regulation of the BsYetJ calcium channel in lipid nanodiscs. Proc Natl Acad Sci U S A. 2020 Dec 1;117(48):30126-30134. PubMed Central PMCID: PMC7720206.

2. **Enhancing Pulsed Dipolar Spectroscopy Resolution for Membrane Protein Studies Using Spin-Labeled Nanodiscs**. Pulsed dipolar spectroscopy (PDS) is a powerful tool for investigating conformational changes in membrane proteins (MPs), but weak dipolar signals often limit its resolution. I explored the use of spin-labeled nanodiscs (NDs) to enhance this resolution. By doping spin-labeled NDs into samples of membrane proteins within unlabeled nanodiscs, I achieved a significant increase in dipolar signal amplitude. This approach enables high-resolution determination of MP structures.

    a. **Li CC**, Hung CL, Yeh PS, Li CE, Chiang YW. Doubly spin-labeled nanodiscs to improve structural determination of membrane proteins by ESR. RSC Adv. 2019 Mar 15;9(16):9014-9021. PubMed Central PMCID: PMC9062051.

3. During my graduate studies, I contributed to various membrane protein studies. This included investigating how Na^+ shifts the conformational equilibrium of a bacterial apical sodium-dependent bile acid transporter (ASBT) using ESR, uncovering the mechanism of photooxidative degradation caused by bacterial translocator protein (TSPO) through spectroscopy-based functional assays and ESR, and demonstrating the critical role of cardiolipin in promoting BAX association and oligomerization using ESR techniques and liposome permeabilization assays.

    a. Yeh PS, **Li CC**, Lu YS, Chiang YW. Structural Insights into the Binding and Degradation Mechanisms of Protoporphyrin IX by the Translocator Protein TSPO. JACS Au. 2023 Oct 23;3(10):2918-2929. PubMed Central PMCID: PMC10598825.
    b. *Lu PH, ***Li CC**, Chiang YW, Liu JH, Chiang WT, Chao YH, Li GS, Weng SE, Lin SY, Hu NJ. Dissecting the Conformational Dynamics of the Bile Acid Transporter Homologue ASBT(NM). J Mol Biol. 2021 Feb 19;433(4):166764. PubMed PMID: 33359100. (*Co-first author)
    c. Lai YC, **Li CC**, Sung TC, Chang CW, Lan YJ, Chiang YW. The role of cardiolipin in promoting the membrane pore-forming activity of BAX oligomers. Biochim Biophys Acta Biomembr. 2019 Jan;1861(1):268-280. PubMed PMID: 29958826.

4. Lipid-dependent temperature sensitivity in a cyclic nucleotide-modulated ion channel. Thermosensation is an ancient sensory process vital for survival, yet the mechanisms by which thermoreceptors sense temperature changes remain elusive, particularly the interactions between thermoreceptors and surrounding lipids. I investigated the temperature-sensing mechanism of SthK, an ion channel from the thermophilic bacterium Spirochaeta thermophila, which can be efficiently expressed in E. coli. As a bacterial homolog of hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels—key regulators of rhythmic activity in the brain and heart that have been suggested as potential cold thermoreceptors in neurons—SthK serve as a promising model for studying thermosensation. Using stopped-flow flux assays, I assessed SthK's temperature sensitivity in controlled lipid compositions and explored lipid-protein interactions via cryo-electron microscopy. Our findings reveal two critical insights: (1) lipid-dependent temperature sensitivity and (2) a potential role for a salt bridge as a temperature sensor.

    a. **Li CC**, NIMIGEAN CM. Mechanism of Lipid-Dependent Thermosensation in an Ion Channel. bioRxiv 2025.06.03.657524; doi: https://doi.org/10.1101/2025.06.03.657524

    b. **Li CC**, NIMIGEAN CM. Mechanism of Lipid-Dependent Cold Sensitivity in the Cyclic Nucleotide-Modulated Ion Channel SthK. Platform presented at: Biophysical Society Meeting; Februrary 2025; Los Angeles, CA

    c. **Li CC**, NIMIGEAN CM. Mechanism of Lipid-Dependent Cold Sensitivity in a Cyclic Nucleotide-Modulated Ion Channel from *Spirochaeta Thermophila*. Lecture presented at: Gordon Research Conference - Ligand recognition and Molecular Gating; March 2024; Ventura, CA

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BIOGRAPHICAL SKETCH

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NAME: Hee-Seop Yoo

eRA COMMONS USER NAME (credential, e.g., agency login): HEE-SEOP

POSITION TITLE: Postdoctoral Associate

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
Ajou University, Suwon, Republic of Korea	B.S.	02/2015	Pharmacy
Ajou University, Suwon, Republic of Korea	Ph.D.	02/2023	Structural Biology
Ajou University, Suwon, Republic of Korea	Postdoc	09/2023	Structural Biology
Weill Cornell Medical College	Postdoc	Present	Anesthesiology

A. Personal Statement

My current research interests are understanding the structure–function relationship of proteins and the mechanisms by which various biomolecules modulate their activity. In 2023, I joined Dr. Crina Nimigean's laboratory at Weill Cornell Medicine, where I have been working on a project titled "Lipid modulation mechanism of large-conductance calcium-activated potassium (BK) channels." BK channels are ubiquitously expressed in human cells and play essential roles in processes such as action potential propagation and muscle contraction.

In this project, I am pursuing two main goals: i) To investigate how BK channels are gated in a voltage-dependent manner within a lipid environment. I successfully expressed full-length tetrameric BK channels and reconstituted them into small unilamellar vesicles. By establishing defined ion gradients, I applied membrane potential and collected initial cryo-EM data, which yielded promising 2D class averages. Additional datasets are being collected to resolve conformational differences between open and closed states.
ii) To elucidate how membrane lipids regulate BK channel activity. Functional assays showed that BK activity is influenced by membrane acyl chain length and cholesterol concentration—key components of native plasma membranes. Cryo-EM with defined lipid compositions will help uncover structural mechanisms behind this regulation.

Access to NCCAT's advanced instrumentation and technical expertise is critical for the success of this project. Given the complexity and physiological importance of the BK channel, high-end cryo-EM tools and expert support are essential. I bring extensive experience in lipoprotein reconstitution and grid preparation, and I am highly motivated to further develop my skills in cryo-EM data acquisition and analysis. This project is well aligned with NCCAT's mission to support cutting-edge cryo-EM research, and I am confident that it will yield valuable insights into the regulation of ion channels in native-like membrane environments.

B. Positions, Scientific Appointments, and Honors

Positions

2023 - present Postdoctoral Associate, Weill Cornell Medical College, New York, NY
2023 - 2023 Postdoctoral Associate, Ajou University, Suwon, South Korea
2023 - 2023 Lecturer, College of Pharmacy, Sookmyung Women's University, Seoul, South Korea
2019 - 2020 Research Scientist, Cellivery Therapeutics Inc., Seoul, South Korea

Honors

2013 Undergraduate Research Award. Ajou University President.
"Structural study on the calcium modulation mechanism of the IP_3 receptor"
2016 Excellent paper award. BK21 Molecular Science & Technology.
"Structural and dynamic insights into the subtype-specific IP_3-binding mechanism of the IP_3 receptor"
2018 JEOL Excellent poster award. Korea Magnetic Resonance Society
"Comparison of IP_3 binding mechanism between IP_3 receptor subtype 1 and 3"
2022 Excellent poster award. Korea Magnetic Resonance Society
"Competitive interactions of Cobll1 and SH3BP1 for PACSIN2 regulate BC transformation and TKI resistance in chronic myeloid leukemia"

C. Contributions to Science

1. Structural and functional analysis of disease-related cellular proteins

My previous research focused on the structural and functional characterization of various cellular proteins using X-ray crystallography. I was also particularly interested in protein-protein interactions related to cell signaling, which I investigated using biophysical techniques such as NMR spectroscopy, isothermal titration calorimetry (ITC), and surface plasmon resonance (SPR). In collaboration with a research team dedicated to therapeutic antibody development and screening, I am currently working on a manuscript that describes the binding mode and structural optimization of antibodies targeting specific colon cancer-related membrane proteins. I believe these studies provide a valuable foundation for understanding the physiological roles of disease-related proteins and for developing therapeutic strategies to target their pathological forms.

I. K. B.*, **Yoo, H. S.***, Oh, C. K.*, Lee, J. R.*, Chung, H. J., Kim, H. N., Kim, S. H., Kee, K. M., Kim, T. Y., Kim, M. S., Kim, B. G., Ra, J. S., Myung, K. J., Kim, H. T., Han, S. H., Seo, M. D., Lee, Y. S. & Kim, D. W. (2022). Reciprocal interactions among Cobll1, PACSIN2, and SH3BP1 regulate drug resistance in chronic myeloid leukemia. *Cancer Medicine*. 10.1002/cam4.4727

II. Lee, S. Y.*, **Yoo, H. S.***, Choi, H. S., Chung, K. Y. & Seo, M. D. (2016). Structural and dynamic insights into the subtype-specific IP3-binding mechanism of the IP3 receptor. *Biochemical Journal*, 473, 3533-3543.

2. Current research on calcium-activated potassium channels

I. **Membrane thickness tunes ball-and-chain inactivation in MthK channels.** The MthK channel, a prokaryotic homolog of the large-conductance calcium-activated potassium (BK) channel from *Methanobacterium thermoautotrophicum*, has been widely used as a model system to study the gating properties of calcium-activated potassium channels. MthK exhibits a ball-and-chain type inactivation mechanism, and our research group previously determined its structural basis using Cryo-EM. In this study, we discovered that the rate of MthK inactivation is modulated by the carbon chain length of membrane lipids. By reconstituting MthK into lipoprotein particles with varying lipid compositions, we demonstrated—using ion flux assays, cryo-EM, and molecular dynamics simulations—that membrane thickness plays a critical role in regulating the inactivation kinetics of the channel. *In manuscript preparation*

II. **Gating mechanism of BK channels in polarized vesicles.** BK channels are large-conductance calcium-activated potassium channels that play essential roles in cellular excitability. To investigate how BK channels respond to membrane potential in a lipid environment, we reconstituted full-length tetrameric BK channels into small unilamellar vesicles and imposed voltage gradients across the membrane using defined ion conditions. Cryo-EM analysis of the proteoliposomes yielded high-quality 2D class averages, suggesting proper incorporation in liposome. Ongoing efforts focus on resolving 3D structures representing different gating states. This work aims to elucidate the voltage-dependent gating mechanism of BK channels in a native-like membrane context.

III. **Lipid modulation of BK channel activity.** BK channel function is known to be sensitive to its lipid environment, but the structural basis of this modulation remains unclear. We investigated how membrane lipid composition regulates BK channel gating by reconstituting the channel into liposomes containing lipids of varying acyl chain lengths and cholesterol concentrations. Functional assays using proteoliposomes revealed that both membrane thickness and cholesterol levels significantly influence

channel activity. These findings suggest that lipid composition can serve as a physiological modulator of BK channels in native membranes. Ongoing cryo-EM studies aim to uncover the structural mechanisms by which specific lipid environments alter channel conformation and gating behavior.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/yoo.hee-seop.1/bibliography/public/

BIOGRAPHICAL SKETCH

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NAME: Zhihan Wang

eRA COMMONS USER NAME (credential, e.g., agency login): WANG.ZHIHAN

POSITION TITLE: postdoctoral associate

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
University of Washington	BS	06/2017	Biochemistry
University of Arizona	PHD	06/2023	Biochemistry and analytical chemistry
Weill Cornell Medical College	Postdoctoral	Present	Anesthesiology

A. Personal Statement

I am molecular biologist with a background of membrane protein application in analytical chemistry and a growing expertise in cryo-EM structural biology. I am committed to integrative approaches that connect structure and function. My career has centered on understanding how membrane proteins like ion channels behave under different conditions such as lipid compositions and temperatures, which naturally requires and extends into high-resolution structural studies. For example, I contributed in a study to investigate the regulation effect of lipid PIP2 on the ion channel SthK. Preliminary functional assays suggested that higher PIP2 content in the lipid composition is correlated to a lower channel open probability, however, little was understood regarding the mechanism on a molecular basis, such as the stoichiometry, where PIP2 bind, and how binding induces activity change. To elucidate the molecular mechanism, we employed cryo-EM to resolve the structural basis of SthK-PIP2 complexes. We also extended the results to a key finding because the structural feature of PIP2 binding, such as the position of the lipid pocket and the corresponding conformation changes that the proteins adopt, can also be observed with other temperature-sensitive ion channels, such as TRP channels. Such findings are exclusively based on high-resolution structural results and are out of the scope of functional assays. Therefore, via the lab that I work at, I am particularly motivated to participate in the BAG program at NCCAT because of the great resource it offers in addressing evolving projects under the goal of elucidating protein activity. Access to regular cryo-EM time through this mechanism would significantly enhance our ability to refine constructs, troubleshoot sample quality, and test hypotheses that address the biophysical properties of the membrane proteins.

B. Positions, Scientific Appointments, and Honors

2023 - present Postdoctoral Associate, Weill Cornell Medical College

C. Contributions to Science

Past experience overview

1. My doctoral research focused on developing biomimetic electrophysiological sensors that are built on polymer scaffold stabilized-black lipid membranes. These sensors were designed to transduce molecular identity and concentration into measurable electrical signals, functionalized by ligand-gated

ion channels reconstituted into artificial lipid bilayers. I explored and developed multiple membrane stabilization methods, including heat-, light-, and chemical-induced polymerizations, allowing for flexible adaptation of these sensors to different conditions. In parallel, I designed chimeric proteins that fused non-channel receptor and channels to expand the range of detectable small molecules. This work cultivated an understanding of membrane protein function, biophysical assay design, and protein-membrane interactions.

   a. **Wang, Z.**; Wang, X.; Zacher, B.; Saavedra, S. S.; Aspinwall, C. A. Effects of Polymer Scaffolds on Electrophysiological Sensors Prepared with Nicotinic Acetylcholine Receptor. (in prep)

2. In addition to sensor work, I characterized small membrane proteins such as viral porins and pore-forming antimicrobial peptides. Collaborating with mass spectrometry, we determined the oligomeric states of these peptides. To assess the functional stoichiometry and pore formation capability, I employed electrophysiology current recording, providing insights beyond the reach of MS alone.

   a. Townsend, J. A.; Fapohunda, O.; **Wang, Z.**; Pham, H.; Taylor, M.; Kloss, B.; Park, S. H.; Opella, S.; Aspinwall, C. A.; Marty, M. T. Differences in Oligomerization of the SARS-CoV-2 Envelope Protein, Poliovirus VP4, and HIV Vpu. *Biochemistry.* 2024, *63* (3), 241–250.
   b. Reid, D. J.; Dash, T.; **Wang, Z.**; Aspinwall, C. A.; Marty, M. T. Investigating Daptomycin–Membrane Interactions Using Native MS and Fast Photochemical Oxidation of Peptides in Nanodiscs. *Anal. Chem.* 2023, *95* (11), 4984–4991.
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Current Cryo-EM structural work

3. As a postdoctoral researcher in the Nimigean lab, I have transitioned my analytical and biophysical background to a more focused protein structure-function research. I contributed to elucidating how the cyclic nucleotide-gated prokaryotic channel SthK interacts with the phospholipid PIP2, integrating lipid modulation into our understanding of channel gating mechanisms. Building on this and other key findings from the lab, we have initiated investigation of thermosensitive TRP channels to investigate how lipid composition alters their temperature dependence. A central hypothesis is that an internal salt bridge forms at a regulatory lipid binding site, which is a feature of SthK that we have resolved at high resolution using single-particle cryo-EM. Through this work, I have gained direct experience in cryoEM sample preparation, biochemical optimization for membrane protein stability, and data collection strategies. My electrophysiological expertise continues to complement and inform construct design and functional validation of cryo-EM samples, enabling us to correlate structural features with biophysical behavior.

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