BIOGRAPHICAL SKETCH

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NAME: Terunaga Nakagawa

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

POSITION TITLE: Associate Professor, Department of Molecular Physiology and Biophysics, Center for Structural Biology, Vanderbilt University, School of Medicine.

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 Tokyo, College of Arts and Sciences	--	1990-1992	Arts and Sciences
University of Tokyo, Graduate School of Medicine	MD	1992-1996	Medicine
University of Tokyo, Graduate School of Medicine	PhD	1996-2000	Molecular Cell Biology and Anatomy, Electron Microscopy
Massachusetts General Hospital, Harvard Medical School	Postdoctoral training	2000-2001	Molecular and Cellular Neuroscience
Massachusetts Institute of Technology	Postdoctoral training	2001-2005	Neuroscience and Molecular Electron Microscopy

A. Personal Statement. My research combines techniques in structural biology, biochemistry, molecular and cellular neurobiology to undercover the molecular basis of ionotropic glutamate receptor (iGluR) function and modulation. As a graduate student I studied kinesin super family (KIF) molecular motors and obtained training in **quick-freeze deep-etch EM**, molecular biology, biochemistry, and cell biology from **Nobutaka Hirokawa** (Univ. of Tokyo). During my postdoctoral training I expanded my expertise in **molecular cellular neuroscience** with Morgan Sheng (MIT) and in **cryo-EM** by conducting experiments in **Thomas Walz**'s laboratory (Harvard). My key paper, "Structures and different conformational states of native AMPAR complexes" (Nature 2005) reported the first low-resolution EM structure and conformational changes of AMPAR obtained from brain. At a resolution that can resolve individual domains, this paper provided a proof-of-principle that full-length AMPARs are tractable target for structural studies, and a significant portion of the reported findings and proposed concepts in this paper remains valid in relation with recent higher-resolution data of recombinant AMPARs reported by others. At UCSD, I focused on investigating the subunit assembly mechanism of iGluRs and the roles of AMPAR auxiliary subunits. In 2012 I moved to Vanderbilt in order to extend my research programs in neuroscience using mouse molecular genetics, high throughput drug screening, electrophysiology, and cryo-EM. My publications were cited in major review articles in the field, including "AMPARs and synaptic plasticity: the last 25 years" (Neuron 2013), authored by Huganir and Nicoll. I continue to be very interested in understanding the mechanism of AMPAR functional regulation by combining approaches in animal models, cryo-EM, biochemistry, molecular biology, light microscopy imaging, and electrophysiology. **I have spent a total of three months during 2016-2017 at MRC-LMB at Cambridge UK** to collaborate on a cryo-EM project with Dr. Ingo Greger. During this opportunity, I was able to advance my knowledge on high-resolution cryo-EM and digital image processing. **As a new direction of our laboratory**, in collaboration with Dr. Julio Cordero-Morales, we started to investigate the structure and mechanism of TRPC channels that mediate metabotropic glutamatergic signaling and vascular regulation. In recent studies, we

obtained multiple cryoEM structures of the TRPC3 and 6 channels at the resolutions ranging from 3.8 to 5.8Å. **I serve as a Scientific Director of Cryo-EM facility at Vanderbilt University Medical School**, which has FEI Polara equipped with K2 Summit Direct Electron Detector among other associated instruments. I am a leading PI, together with five other structural biology PIs at Vanderbilt, of a Trans Institutional Programs (TIPs) Award aimed to establish a new cryo-EM facility that will house the Titan Krios equipped with a K3 Detector, an energy filter, Falcon3 detector, and a phase plate, which will arrive in late 2018 and set up in mid 2019.

B. Positions and Honors

Positions and Employment

1997-2000	Predoctoral fellow, Graduate School of Medicine, University of Tokyo, Tokyo, Japan, with Professor Nobutaka Hirokawa.
2000-2001	Postdoctoral fellow, Massachusetts General Hospital, Harvard Medical School, with Professer Morgan Sheng.
2001-2005	Postdoctoral fellow, Massachusetts Institute of Technology, with Professor Morgan Sheng.
2005-2012	Assistant Professor, Department of Chemistry and Biochemistry, University of California, San Diego.
2012-present	Associate Professor, Department of Molecular Physiology and Biophysics, Center for Structural Biology, Vanderbilt University, School of Medicine.

Other Experience and Professional Memberships

Member, Society for Neuroscience
Member, AAAS

2012-2016	Co-Scientific Director, Cryo-EM facility, Vanderbilt University
2013-14	Ad hoc grant reviewer for Medical Research Council, UK
2014	Ad hoc grant reviewer for NIH study section, Biophysics of Neural Systems (BPNS)
2014	Ad hoc grant reviewer for European Research Council, EU
2014-present	Executive Committee, Center for Structural Biology, Vanderbilt University
2015-16	Ad hoc grant reviewer for NIH study section, NST-2 (NINDS, F30, K99)
2016 and 2018	Ad hoc grant reviewer for NIH study section, SYN
2016-present	Editorial board, Journal of Biological Chemistry
2016	Ad hoc grant reviewer for NIH special emphasis panel.
2017-present	Scientific Director, Cryo-EM facility, Vanderbilt University

Honors

1) Hajime Hagiwara travelling fellowship (DNAX research institute, Palo Alto, CA, USA) (1993)
2) Japan Society for the Promotion of Science Research Fellowship for Young Scientists. (1997-2000)
3) Human Frontier Science Program Long-term Fellowship. (2000-2003)
4) Howard Hughes Medical Institute postdoctoral research associate. (2003-2005)
5) Hellman Faculty Fellow Award (2007)
6) John Merck Fund Faculty Award (2007-2010)
7) NARSAD Young Investigator Award (2008-2009)
8) Kazato Prize in Electron Microscopy (2012)
9) NARSAD Independent Investigator Grant (2017-2019)

C. Contribution to science

1. Structures of ionotropic glutamate receptors

I have contributed to understanding the molecular architecture of the ionotropic glutamate receptors by using single particle electron microscopy and X-ray crystallography. My key paper, “Structures and different conformational states of native AMPAR complexes” (Nature 2005) reported the first low-resolution structure

and global conformational changes of heterotetrameric AMPAR obtained from brain. This is the first and the only structure of AMPARs that were obtained from the brain tissue. At a resolution that can resolve individual domains, this paper provided a proof-of-principle that full-length AMPARs are tractable target for structural studies. A significant portion of the reported findings and proposed concepts in this paper remains valid in relation to recent higher-resolution data of recombinant AMPARs reported by Gouaux, Walz, Mayer, Sobolevsky, Frank, and Subramaniam. In addition to the conformational changes in desensitized AMPARs that are characterized by displacements of the NTD dimers at varying degrees, we also found tight association of TARP family of proteins with the brain AMPARs. Until this paper TARPs were thought to be dynamic interactors that only transiently interact with the core ion channel. This paper together with a paper from Nicoll lab (Vandenberghe et al 2005) contributed the concept of auxiliary subunits in AMPARs. We have continued on this line of research. In brief, in 2010 we were the first group to investigate the structures of subunit assembly intermediate of AMPARs using single particle EM. In the same study, we found that auxiliary subunit stargazin preferentially interacts with the tetrameric AMPARs and not with the assembly intermediates. We reported the first X-ray structure of the isolated GluN1-NTD in 2011, a study that lead to proposing molecular pathways of NMDAR subunit assembly. The global assembly of NMDAR we proposed was consistent with the recent X-ray crystal structures of the full-length NMDAR published by Furukawa and Gouaux. We have also contributed to understanding the structures of the trans synaptic complexes formed by GluD1/2-cbln1-neurexin.

1) Elegheert J, Kakegawa K, Clay JE, Shanks NF, Behiels E, Matsuda K, Kohda K, Miura E, Rossmann M, Mitakidis N, Motohashi J, Chang VT, Siebold C, Greger IH, **Nakagawa T**, Yuzaki M, Aricescu AR, Structural Paradigm for Integration of GluD Receptors within Synaptic Organizer Complexes **Science 2016** Jul 15;353(6296):295-9. PMID: 27418511: PubMed Central PMC5291321.
2) Farina AN, Blain KY, Maruo T, Kwiatkowski W, Choe S, **Nakagawa T**. Separation of domain contacts is required for heterotetrameric assembly of functional NMDA receptors. **J Neurosci. 2011** Mar 9;31(10):3565-79. PubMed PMID: 21389213; PubMed Central PMCID: PMC3063151.
3) Shanks NF, Maruo T, Farina AN, Ellisman MH, **Nakagawa T**. Contribution of the global subunit structure and stargazin on the maturation of AMPA receptors. **J Neurosci. 2010** Feb 17;30(7):2728-40. PubMed PMID: 20164357; PubMed Central PMCID: PMC2842908.
4) **Nakagawa T**, Cheng Y, Ramm E, Sheng M, Walz T. Structure and different conformational states of native AMPA receptor complexes. **Nature. 2005** Feb 3;433(7025):545-9. PubMed PMID: 15690046.

2. Identification of new AMPAR auxiliary subunits and mechanism of auxiliary subunit action on AMPARs.

The AMPAR function is amplified when AMPAR associate with various auxiliary subunits that are known to modulate the ion channel in diverse ways. To improve our understanding on the mechanism and function of auxiliary subunits in the brain, in 2012, the year when we moved to Vanderbilt, we published a key paper in Cell Reports reporting the comparative proteomics of AMPAR and kainate receptor in brain, from which we identified a new AMPAR auxiliary factor GSG1L. GSG1L has a unique function in delaying the AMPAR recovery from desensitization at a magnitude grater than any of the known auxiliary subunit. In 2014, we defined sub-regions in CNIH-3 auxiliary subunit and AMPAR required for binding and gating modulating, and in the same paper reported methods to prepare recombinant AMPAR-auxiliary subunit complexes for structural studies.

1) Hawken NM, Zaika EI, **Nakagawa T.** Engineering defined membrane-embedded elements of AMPA receptor induces opposing gating modulation by CNIH3 and stargazin **J Physiol. 2017** Oct 15;595(20):6517-6539. doi: 10.1113/JP274897. Epub 2017 Sep 12. PubMed PMID: 28815591; PubMed Central PMCID: PMC5638889.
2) Azumaya CM, Days EL, Vinson PN, Stauffer S, Sulikowski G, Weaver CD, **Nakagawa T.** Screening for AMPA receptor auxiliary subunit specific modulators. **PloS one. 2017**; 12(3):e0174742. PubMed [journal] PMID: 28358902, PMCID: PMC5373622.
3) Shanks NF, Cais O, Maruo T, Savas JN, Zaika EI, Azumaya CM, Yates JR 3rd, Greger I, **Nakagawa T.** Molecular Dissection of the Interaction between the AMPA Receptor and Cornichon Homolog-3. **J Neurosci. 2014** Sep 3;34(36):12104-20. PubMed PMID: 25186755; PubMed Central PMCID: PMC4152608.
4) Shanks NF, Savas JN, Maruo T, Cais O, Hirao A, Oe S, Ghosh A, Noda Y, Greger IH, Yates JR 3rd, **Nakagawa T**. Differences in AMPA and Kainate Receptor Interactomes Facilitate Identification of

AMPA Receptor Auxiliary Subunit GSG1L. **Cell Rep. 2012** Jun 28;1(6):590-8. Epub 2012 May 23. PubMed PMID: 22813734; PubMed Central PMCID: PMC3401968.

3. Molecular architectures and mechanisms of molecules utilized in synaptic plasticity

The postsynaptic density is the site where macromolecular assembly of receptors, cell adhesion proteins, and scaffold protein mediate key biological functions in synaptic plasticity, transmission, maintenance, and dynamics. I have been interested in studying the molecular mechanism of synaptic plasticity and contributed to visualizing the first molecular architecture single particle EM images of RPTPσ, alpha neurexin, *Botullinum* neurotoxin E, PSD-95, and SAP97. The structures obtained lead to functional models that were tested in each study (except for the study on *Botullinum* neurotoxin E).

1) Coles CH, Mitakidis N, Zhang P, Elegheert J, Lu W, Stoker AW, **Nakagawa T**, Craig AM, Jones EY, Aricescu AR. Structural basis for extracellular cis and trans RPTPσ signal competition in synaptogenesis. **Nat Commun. 2014** Nov 11;5:5209. doi: 10.1038/ncomms6209. PubMed PMID: 25385546; PubMed Central PMCID: PMC4239663.
2) Comoletti D, Miller MT, Jeffries CM, Wilson J, Demeler B, Taylor P, Trewhella J, **Nakagawa T**. The macromolecular architecture of extracellular domain of alphaNRXN1: domain organization, flexibility, and insights into trans-synaptic disposition. **Structure. 2010** Aug 11;18(8):1044-53. PubMed PMID: 20696403; PubMed Central PMCID: PMC2948785.
3) Fischer A, Garcia-Rodriguez C, Geren I, Lou J, Marks JD, **Nakagawa T**, Montal M. Molecular architecture of botulinum neurotoxin E revealed by single particle electron microscopy. **J Biol Chem. 2008** Feb 15;283(7):3997-4003. Epub 2007 Nov 20. PubMed PMID: 18032388.
4) **Nakagawa T**, Futai K, Lashuel HA, Lo I, Okamoto K, Walz T, Hayashi Y, Sheng M. Quaternary structure, protein dynamics, and synaptic function of SAP97 controlled by L27 domain interactions. **Neuron. 2004** Oct 28;44(3):453-67. PubMed PMID:15504326.

4. Identification and molecular mechanism of KIF molecular motor proteins

As a graduate student I studied kinesin super family molecular motors and obtained training in quick-freeze deep-etch EM, molecular biology, biochemistry, and cell biology from Nobutaka Hirokawa (Univ. of Tokyo). I have contributed to identification of nearly 1/3 of the KIF (kinesin super family) motor proteins, many of which are utilized in various cellular functions in vesicle trafficking. I have also contributed to identifying the in vivo function and mechanism of vesicle cargo recognition of the KIFs, some of which were published in the top journals. The key finding is that the KIF motors use adaptor and scaffold protein in order to associate with membrane proteins that are part of the cargo of transporting vesicles they carry.

1) Zhao C, Takita J, Tanaka Y, Setou M, **Nakagawa T**, Takeda S, Yang HW, Terada S, Nakata T, Takei Y, Saito M, Tsuji S, Hayashi Y, Hirokawa N. Charcot-Marie-Tooth disease type 2A caused by mutation in a microtubule motor KIF1Bbeta. **Cell. 2001** Jun 1;105(5):587-97. PubMed PMID: 11389829.
2) **Nakagawa T**, Setou M, Seog D, Ogasawara K, Dohmae N, Takio K, Hirokawa N. A novel motor, KIF13A, transports mannose-6-phosphate receptor to plasma membrane through direct interaction with AP-1 complex. **Cell. 2000** Nov 10;103(4):569-81. PubMed PMID: 11106728.
3) Setou M, **Nakagawa T**, Seog DH, Hirokawa N. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. **Science. 2000** Jun 9;288(5472):1796-802. PubMed PMID: 10846156.
4) **Nakagawa T**, Tanaka Y, Matsuoka E, Kondo S, Okada Y, Noda Y, Kanai Y, Hirokawa N. Identification and classification of 16 new kinesin superfamily (KIF) proteins in mouse genome. **Proc Natl Acad Sci U S A. 1997** Sep 2;94(18):9654-9. PubMed PMID: 9275178; PubMed Central PMCID: PMC23244.

5. High resolution cryo-EM analyses of ion channel architecture (TRPC and IP3R)

I am also interested in investigating ion channels other than the iGluRs. My goal is to broaden my views to diverse molecular entities so that common and different mechanism between ion channel members could be understood. In collaboration with Dr. Julio Cordero-Morales (University of Tennessee Health Science Center), we study the structure and gating mechanism of on the TRPC channels. We have recently obtained a 3.8Å resolution cryo-EM structure of the cytoplasmic domain of the TRPC6, where many human disease-causing mutations accumulate. Our study generated atomic model of this domain and conceptual framework to classify

these mutants for further electrophysiological investigation. In collaboration with Erkan Karakas (Vanderbilt) we study the IP3 receptors using cryo-EM.

1) Azumaya CM, Sierra-Valdez F, Cordero-Morales JF, **Nakagawa T**. Cryo-EM structure of the cytoplasmic domain of murine transient receptor potential cation channel subfamily C member 6 (TRPC6). **J Biol Chem. 2018** May 11. PubMed PMID: 29752403. PMCID: PMC6028952.
2) Sierra-Valdez F*, Azumaya CM*, Romero LO, **Nakagawa T**#, and Cordero-Morales JF#. **(# co-correspondence authors**, * co-first authors) Structural and functional analyses of TRPC3 reveal allosteric gating modulation by the cytoplasmic domain. **J Biol Chem. 2018** Aug 23. PubMed PMID: 30139744. PMCID: in process.
3) Azumaya CM, Linton EA, **Nakagawa T**#, and Karakas E#. **(# co-correspondence authors)** Structural basis for the competitive inhibition of inositol triphosphate receptors by EDTA (in review)

Total number of publications: 35 Articles in PubMed. The following link provides the complete list of all my PubMed listed papers published to date:
http://www.ncbi.nlm.nih.gov/sites/myncbi/terunaga.nakagawa.1/bibliograpahy/40638054/public/?sort=date&direction=descending

D. Research Support.

Active:

1) NARSAD Independent Investigator Award (PI: Terunaga Nakagawa) 09/01/2017-08/31/2019
Title: Modulation of excitatory synaptic transmission in mental illnesses
Our goal is to provide a molecular explanation for a concept that abnormal communication between neurons is an underlying mechanism for various mental illnesses, such as ASD and major depression disorders.

In review or to be resubmitted:

1) NIH R01 (PI: Terunaga Nakagawa) To be resubmitted
Title: Regulation of synaptic function by GSG1L, a unique regulator of AMPAR function
GSG1L is enriched in adults and changes synaptic function in a previously unexpected way by age-dependently negatively controlling AMPARs. We will investigate its mechanism for action and its role in synaptic function and behaviors.

2) NIH R01 (MPI: Julio Cordero-Morales, Terunaga Nakagawa) 04/01/2019-03/31/2024
Title: Structural and functional mechanism of TRPC channels
Members of the canonical transient receptor potential (TRPC) ion channel subfamily act as biological sensors by decoding physiological cues in the environment to regulate cellular function. Our objective is to establish the molecular bases underlying TRPC channel activation and modulation using structural (cryo-EM) and functional (electrophysiology) approaches.

Completed:

1) R01HD061543-01A2 (PI: Terunaga Nakagawa). 07/10/2010-05/31/2016 (no-cost extension),
Eunice Kennedy Shriver National Institute of Child health and Development
Title: Molecular Anatomy of Mature and Immature Glutamate Receptors
By combining structural biological and cell biological approaches, this project aims to determine the molecular mechanisms of ionotropic glutamate receptor assembly, function, and modulation.
2) R01MH091664 (PI: Terunaga Nakagawa). 07/15/2010-04/30/2014, (no cost extension)
National Institute of Mental Health
Title: Isolation of ribonucleic acids that are attached to the neuronal membrane
This EUREKA project aims to identify novel RNAs that regulate membrane function in the nervous system.
3) R21 MH102546 (PI: Terunaga Nakagawa, co-PI: David Weaver). 08/01/2014 - 07/31/2016,
National Institute of Mental Health
Title: Identifying molecules that modulate auxiliary factors of AMPA receptors
Using a cell base assay in a high throughput screening (HTS) format, this project aims to identify new chemical probes that modulate the activity of AMPAR auxiliary subunits.

BIOGRAPHICAL SKETCH

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NAME: Cordero-Morales, Julio Francisco

eRA COMMONS USER NAME (credential, e.g., agency login): JULIOC1

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
Universidad Central de Venezuela	Licentiate in Biology	01/2001	Cell Biology
University of Virginia	Ph.D.	05/2008	Physiology
University of California, San Francisco	Postdoctoral	02/2014	Physiology

A. Personal Statement

I am a electrophysiologist and biochemist whose current research interests pertain to the structure and function of ion channels involved in blood pressure regulation and somatosensation. My graduate training was in biophysics; under the supervision of Dr. Eduardo Perozo I worked to provide a physical framework for understanding inactivation in K^+ channels, using patch clamp, electron paramagnetic resonance spectroscopy (EPR) and X-ray crystallography. During my postdoctoral research, I studied the mechanism by which transient receptor potential (TRP) channels enhance neuronal activity, under the supervision of Dr. David Julius. My combined background and expertise in electrophysiology, biochemistry, structural biology, and sensory physiology make me uniquely qualified to tackle fundamental questions and to develop multi-disciplinary approaches to decipher structure-function relationships of ion channels at the molecular level.

My current research focuses on investigating the mechanisms by which polymodal receptors translate various stimuli into protein function. During my first four years as an Assistant Professor I was able to combine different approaches to determine how fatty acid and lipids modulate the function of TRP channels. Moreover, I have generated tools to express, purify, and spin label TRP channels for EPR and double electron-electron resonance (DEER) spectroscopies. This work represents the initial step towards using spectroscopic methods to understand the polymodal gating of this ion channels family. I have also integrated cryoEM in our research to establish the molecular bases underlying TRPC channels activation and modulation by bioactive lipids. During this period, I have trained three postdoctoral fellows that have taken positions in industry and academia. Regarding my service, I am a regular member of the AHA peer review group and I have been participating in Biophysical Society committees, such as postdoc to faculty Q&A, networking mentor volunteers, and PI to PI meeting.

B. Positions and Honors

Positions and Employment

1998-1999 Research Assistant, Laboratory of Structural Biology, Instituto Venezolano de Investigaciones Científicas, Centro de Biofísica y Bioquímica, Caracas, Venezuela. Mentor: Dr. Raul Padron.

1999- 2001 Research Assistant, Laboratory of Membrane Physiology, Universidad Central de Venezuela, Instituto de Biología Experimental (I.B.E), Caracas, Venezuela. Mentor: Dr. Pedro Romero.

2001-2004 Research Assistant, Department Molecular Physiology and Biological Physics, The University of Virginia, Charlottesville, VA. Mentor: Dr. Eduardo Perozo.

2004-2008 Graduate Student, Department Molecular Physiology and Biological Physics, The University of Virginia, Charlottesville, VA. Mentor: Dr. Eduardo Perozo.

2008-2009 Research Associate, Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL. Mentor: Dr. Eduardo Perozo.

2009-2014 Postdoctoral Fellow, Department of Physiology, University of California, San Francisco, CA. Mentor: Dr. David Julius.

2014-present Assistant Professor, Department of Physiology, The University of Tennessee Health Science Center, Memphis, TN.

Honors

2007 Outstanding Graduate Student Award, University of Virginia Structural, Computational Biology and Biophysics.

2008 Student Research Achievement Award 52nd Annual Meeting of the Biophysical Society.

2011-2014 Lilly Research Laboratories Fellow of the Life Sciences Research Foundation

Professional Memberships and Service

2001- Member, Biophysical Society.

2001- Member, Sociedad de Biofísica Latinoamericana (SOBLA).

2011- Member, Society for Neuroscience.

2014- Member of the Neuroscience Institute at The University of Tennessee Health Science Center

2014- Member, American Pain Society.

2014- Member, American Heart Association.

2014- Invited reviewer: Nature communications, Biophysical Journal, Journal of Neuroscience and Journal of General Physiology

2015- Member, The Dean's Faculty Advisory Committee. University of Tennessee.

2017- Member, Society of General Physiologists.

2018- Member, American Physiological Society.

C. Contribution to Science

1. Inactivation at the potassium channel selectivity filter. One of the fundamental challenges in the ion channel field is to understand the molecular mechanism of potassium channel inactivation. Inactivation is a widespread process by which a conducting ion channel enters a stable non-conducting state. Many human diseases are due to alterations in the inactivation (e.g., cardiac arrhythmias), making this process relevant to human health. To understand the inactivation process in potassium channels, I carried out a multidisciplinary approach using mutagenesis, protein biochemistry and crystallization, electron paramagnetic resonance spectroscopy, and electrophysiological analysis. By performing an alanine scan in the region of the channel that forms the pore helix and the external vestibule, I identified several residues essential for the inactivation process in the prokaryotic (KcsA) and eukaryotic potassium channel (Kv1.2). I demonstrated that interactions between these residues at the selectivity filter, pore helix, and adjacent external vestibule promote and modulate channel entry into the inactivated state. Collectively, these results provide a framework for understanding the molecular mechanism of channel inactivation and could shed light on the mechanisms involved in heart arrhythmia.

a) **Cordero-Morales J.F**, Cuello L, Zhao Y, Jogini V, Cortes D.M, Roux B, and Perozo E (2006). Molecular determinants of gating at the potassium channel selectivity filter. *Nature Structural and Molecular Biology* 13: 311-318 (Journal Cover). **PMID number: 16532009.**
b) **Cordero-Morales J.F**, Cuello L, and Perozo E (2006). Voltage-dependent gating at the potassium channel selectivity filter. *Nature Structural and Molecular Biology* 13: 319-321. **PMID: 16532008**.
c) **Cordero-Morales J.F**, Jogini V, Lewis A, Vásquez V, Cortes D.M, Roux B, and Perozo E (2007). Molecular driving forces determining potassium channel slow inactivation. *Nature Structural and Molecular Biology* 14: 1062-1069. **PMID number: 17922012.**
d) Chakrapani S*, **Cordero-Morales JF***, Jogini V, Pan A, Cortes DM, Roux B and Perozo, E (2011). On the structural basis of modal gating behavior in K+ channels. *Nature Structural and Molecular Biology* 18:67-75. *Denotes equal contribution. **PMCID: PMC3059741.**

2. Molecular basis of physical and chemical stimuli detection by sensory receptors. Transient receptor potential vanilloid 1 (TRPV1) is a channel activated by noxious heat and inflammatory agents that contribute to pain hypersensitivity. It is unknown how TRPV1 stimuli sensitivity thresholds are set and how these thresholds are altered during injury. We rationalized that the analysis of TRPV1 orthologs with distinct biophysical properties would uncover mechanisms relevant to the human-counterpart proteins. In this work, I carried out the functional characterization of the TRPV1 orthologs using patch clamp and two-electrode voltage clamp. I demonstrated that through alternative splicing of TRPV1 transcripts, vampire bats produce a channel with a truncated carboxy-terminal cytoplasmic domain that has a lower thermal activation threshold (30°C) than its human counterpart (42°C); hence, vampire bats' C-terminal domain tunes thermal sensitivity. Similarly, in humans, membrane bioactive lipids regulate TRPV1 sensitivity to thermal stimuli through interaction with its C-terminal domain. Therefore, these results implicate the C-terminal domain as a clinically important target region for generating novel therapeutic agents to modulate TRPV1 stimuli sensitivity thresholds.

The human TRPA1 (hTRPA1) channel is the target of environmental irritants and proalgesic agents that depolarize sensory neurons to intensify inflammatory pain, whereas the rattlesnake ortholog channel is barely sensitive to these environmental cues. I reasoned that chimeras generated among these orthologs with distinct functional properties could help delineate channel domains that specify stimulus detection. Using a structure-function approach, I determined that sensitivity to physical (e.g., heat) and chemical stimuli (e.g., mustard oil and calcium) is localized within the N-terminal domain, and identified this region as an integrator of diverse physiological signals that regulate sensory neuron excitability. These results represented the first demonstration that stimulus sensitivity can be faithfully and precisely transferred from TRPA1 orthologs.

a) Gracheva EO*, **Cordero-Morales* JF***, González-Carcacía JA, Ingolia NT, Aranguren CI, Manno C, Weissman JS and David Julius (2011). Ganglion-specific splicing of TRPV1 underlies infrared sensation in vampire bats. *Nature*: 476(7358): 88-91 (Journal Cover). **PMCID: PMC3535012**. *Denotes equal contribution.
b) **Cordero-Morales JF***, Gracheva EO* and Julius D (2011). Cytoplasmic ankyrin repeat domains of TRPA1 dictate sensitivity to thermal and chemical stimuli. *PNAS 108: E1184-E1191.* *Denotes equal contribution. **PMCID: PMC3219143.**

3. The Role of Bioactive Lipids in Transient Receptor Potential Channels Gating. TRPV1 is a sensory receptor activated by noxious heat (>42°C), capsaicin (the pungent ingredient of "hot" chili peppers), toxins, and proalgesic inflammatory agents (e.g., extracellular protons and bioactive lipids) that are produced in response to tissue injury. Therefore, as a polymodal receptor, TRPV1 represents a potential target for treating a variety of pain states. Whether TRPV1 is directly or indirectly activated by heat and chemical stimuli was a topic of debate. By reconstituting purified TRPV1 into liposomes, I showed that TRPV1 channels are intrinsically heat-sensitive and negatively regulated by phosphoinositides. I also demonstrated that association of the TRPV1 C terminus with the membrane influences channel sensitivity to chemical and physical stimuli. This work is consistent with a model in which phosphoinositide turnover enhances TRPV1 sensitivity to other stimuli, contributing to thermal hyperalgesia.

a) Cao E, **Cordero-Morales JF**, Liu B, Qin F, and Julius D (2013). TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids. *Neuron* 77:667-679. **PMCID: PMC3183019.**

Based on my background in ion channel biophysics and physiology, ***I decided to focus on understanding the polymodal gating mechanism of TRP channels for my independent career.*** Dietary consumption of ω-3 polyunsaturated fatty acids (PUFAs), present in fish oils, is known to improve the vascular response, but their molecular targets remain largely unknown. TRPV4 is a polymodal ion channel that plays a role in reducing systemic blood pressure by integrating hemodynamic forces and chemical cues in endothelial and smooth muscle cells. Using an in vivo screening in a transgenic worm expressing rat TRPV4, together functional and biophysical approaches, we demonstrated ω-3 PUFAs enhance TRPV4 function in human endothelial cells and proposed a molecular framework for understanding the beneficial effects of fatty acids in the vascular system.

a) Caires R, Sierra-Valdez F, Millet JRM, Herwig JD, Roan E, Vásquez V, **Cordero-Morales JF**. 2017. Omega-3 Fatty Acids Modulate TRPV4 Function Through Plasma Membrane Remodeling. ***Cell Reports***, 21: 246-258. **PMID: 28978477**

b) **Cordero-Morales JF** and Vásquez V. 2018. How membrane lipids contribute to ion channels function, a fat perspective on direct and indirect interactions. ***Current Opinion in Structural Biology***. Invited review. 27; 51:92-98. **PMID: 29602157**

4. Structural Dynamics of Transient Receptor Potential Channels. I took the challenge of implementing spectroscopic approaches in mammalian ion channels. Functional and structural characterizations of TRPV1 shed light on vanilloid activation, yet the mechanisms for temperature and proton gating remain largely unknown. Spectroscopic approaches are needed to understand the mechanisms by which TRPV1 translates diverse stimuli into channel opening. We have contributed by engineering a minimal cysteine-less rat TRPV1 construct that can be stably purify and reconstituted for EPR and DEER spectroscopy measurements. Moreover, we determined that S5-pore helix loop influences protein stability and vanilloid and proton responses, but not thermal sensitivity. Our work represents the initial step towards using EPR and DEER methods in TRP channels to measure mobilities and distance changes associated with polymodal gating.

a) Velisetty P, Stein RA, Sierra-Valdez F, Vasquez V, **Cordero-Morales JF**. 2017. Expression and Purification of the Pain Receptor TRPV1 for Spectroscopic Analysis. ***Scientific Reports***, 7: 9861. **PMCID: PMC5575240.**

b) Sierra-Valdez F, Stein RA, Velissety P, Vásquez V, **Cordero-Morales JF**. 2018. Expression and Purification of the Pain Receptor TRPV1 for Spectroscopic Analysis. ***Jove***.

5. Structural and Functional Analysis of Canonical Transient Receptor Potential Channels. The canonical transient receptor potential 6 (TRPC6) ion channel is expressed in the podocyte and mutations in its cytoplasmic domain cause progressive kidney failure and focal segmental glomerulosclerosis (FSGS) in humans. We have contributed with a cryoEM structure of the cytoplasmic domain of murine TRPC6 at 3.8Å resolution. We highlight that multiple FSGS mutations converge at the buried interface between the vertical coiled-coil and the ankyrin repeats, which form the dome, suggesting these regions are critical for allosteric gating modulation.

a) Azumaya CM, Sierra-Valdez F, **Cordero-Morales JF***, and Nakagawa T*. 2018. Cryo-EM structure of TRPC6 cytoplasmic domain. *JBC*. **PMID: 29752403** * Corresponding author.

The canonical transient receptor potential channel member 3 (TRPC3) acts as a biological sensor by decoding physiological cues in the environment to regulate cellular function, including neuronal excitability and blood pressure. Malfunction of TRPC3 has been associated with neurodegenerative diseases, memory loss, and hypertension. We have contributed with cryoEM structures of the full-length and the cytoplasmic domain of TRPC3 in the apo state at 5.8 Å and 4.0 Å resolution. Using structural and functional analysis, we propose a model in which conformational changes in this helix have a direct impact in channel gating.

a) Sierra-Valdez F, Azumaya CM, Romero LO, Nakagawa T*, **Cordero-Morales JF***. Structure-function analyses of the ion channel TRPC3 reveal that its cytoplasmic domain allosterically modulates channel gating. ***J Biol Chem***. Aug 23.

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D. Research Support

Ongoing

NIH - 1R01GM125629-01 Cordero-Morales (PI) 01/01/18-12/31/22
National Institutes of Health (NIGMS)
The role of bioactive lipids in Transient Receptor Potential Channels Gating

Complete

AHA 15SDG25700146 Cordero-Morales (PI) 07/01/15-06/30/18
American Heart Association, Science Development Grant (Greater Southeast).
The goal of this study is to elucidate the mechanism of TRP4 activation and its role in vascular function.