BIOGRAPHICAL SKETCH

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NAME: Chakrapani, Sudha

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

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)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Madras, Chennai, India	B.S.	06/1995	Chemistry
University of Pune, India	M.S.	06/1997	Biochemistry
Indian Institute of Technology, India	M.Tech.	02/1999	Biomedical Engineering
University at Buffalo, Buffalo, NY	Ph.D.	05/2004	Physiology & Biophysics
University of Virginia, Charlottesville, VA	Postdoctoral	01/2006	Physiology & Biophysics
University of Chicago, Chicago, IL	Postdoctoral	07/2008	Physiology & Biophysics

A. Personal Statement

My long-standing scientific interest has been in developing a molecular-level understanding of ion-transport phenomenon across cellular membranes that occurs under normal and pathophysiological conditions. My research over the last 20 years has focused on ion channels that mediate fast synaptic transmission at the neuronal and neuromuscular junction; namely, ligand- and voltage- gated ion channels. My scientific approach is a combination of cutting-edge multidisciplinary tools that includes Cryo-EM and X-ray crystallography for high-resolution structure determination, EPR spectroscopy for protein dynamic measurements, and electrophysiology for functional characterization of ion channels.

We solved the first cryo-EM structure of the full-length 5-HT_{3A}R channel in its resting conformation (*Nature Communications, 2018*), in serotonin-bound states that revealed the conformational changes underlying channel activation (*Nature, 2018*), bound to setrons, clinically used drugs in the treatment of nausea and vomiting in patients undergoing cancer treatments, revealing the mechanism of setron-mediated inhibition in these channels (*Nature Communications, 2019; eLife 2020*). Together, these structures represent the first set of gating conformational states along the ligand-driven activation pathway described for a full-length pentameric-ligand gated ion channel (pLGIC). We recently developed a comprehensive structural scheme of glycine receptors gating by determining cryo-EM structures of the channel in the apo, open, and desensitized conformations in a lipid bilayer environment (*Nature Communications*, 2020). In summary, with the experience I have gained in diverse structural, dynamics, and functional approaches, and further equipped with the cutting-edge cryo-EM technique, we are now poised to address some of the fundamental questions in the membrane protein field that have remained elusive so far.

Teaching and Mentoring: I have been extremely fortunate to work with and mentor extremely talented individuals who have helped build my research program and are an integral part of what we have achieved as a team. In the last 10 years, *I have trained 7 graduate students (past and current) of which 5 are female.* All my predoctoral trainees and postdoctoral trainees have remained in biomedical science professions. Among past trainees from the lab, two of the three predoctoral trainees hold leadership positions in pharmaceutical industries and two of the three postdoctoral trainees are independent PIs with faculty positions. In addition, I have participated in 24 graduate student thesis progress committees, of which I am Chair on 7 of them. I am

on the mentoring Committee for 6 Junior faculty members to provide them guidance on the grants, tenure, promotion, and professional growth. Since 2012, I serve on the Graduate Education Committee at the Department of Physiology and Biophysics and in 2019 was appointed to the MSTP Steering Committee. Among the accolades won by my trainees, the notable ones include a postdoctoral fellowships from the American Heart Association by Dr. Basak, and Dr. Arvind Kumar; Biophysical Society Student Travel Award and the University of Chicago postdoctoral Fellowship by Dr. Nicholas Schmandt, and the *Recknagel Award* from the DPB by Ms. Yvonne Gicheru and Ms. Kayla Kindig. Over the last ten years my lab has hosted nine students from the DPB Summer Undergraduate Research Program and the Heart Lung Blood Summer Research Program. Two of these students, Ross Bonner and Lauren Talley (URM), are contributing authors on a paper in *Journal of General Physiology, 2015*.

Citations:

1. Basak S, Kumar A, Ramsey S, Gibbs E, Kapoor A, Filizola M, Chakrapani S. High-resolution structures of multiple 5-HT3AR-setron complexes reveal a novel mechanism of competitive inhibition. **eLife**. 2020;9. Epub 2020/10/17. doi: 10.7554/eLife.57870. PubMed PMID: 33063666.
2. Kumar A, Basak S, Rao S, Gicheru Y, Mayer ML, Sansom MSP, **Chakrapani S*.** (2020) Mechanisms of activation and desensitization of full length glycine receptors in lipid nanodisc. ***Nature Communications*** Jul 27;11(1):3752. doi: 10.1038/s41467-020-17364-5.PMID: 32719334
3. Basak S, Gicheru Y, Rao S, Sansom MSP, **Chakrapani S***. (2018) Cryo-EM reveals two distinct serotonin-bound conformations of full-length 5-HT3A receptor. ***Nature***.;563(7730):270-4. doi: 10.1038/s41586-018-0660-7. PubMed PMID: 30401837; PMCID:PMC6237196 (*Article Recommended by Faculty 1000)*
4. Schmandt, N., P. Velisetty, S. V. Chalamalasetti, R. A. Stein, R. Bonner, L. Talley, M. D. Parker, H. S. McHaourab, V. C. Yee, D. T. Lodowski and **S. Chakrapani*** (2015). "A chimeric prokaryotic pentameric ligand-gated channel reveals distinct pathways of activation." J Gen Physiol **146**(4): 323-340.

Ongoing and recently completed projects that I would like to highlight include:

NIH R35 GM134896
Chakrapani (PI)
01/01/20 – 12/31/24
Structure and Function of Pentameric Ligand-Gated Ion Channels

Completed Research Support
NIH R01 GM131216
Chakrapani (PI)
01/1/19 – 12/31/22
Structure, Function, and Modulation of Serotonin (3A) receptors"
(Rolled into R35 MIRA Award).

NIH R01 GM108921
Chakrapani (PI)
09/1/14 – 08/31/20
Molecular Mechanisms of Desensitization and Drug Modulation in Ligand-Gated Ion Channels.
(Renewal funded as R35 MIRA Award)

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2020-present Professor, Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH
2020-present Director, Cleveland Center for Membrane and Structural Biology

2018-present Director, Cryo-Electron Microscopy Core, Case Western Reserve University, Cleveland, OH

2017-2020 Associate Professor (Tenured), Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH

2010-2017 Assistant Professor (Tenure-track), Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH

2008-2010 Research Assistant Professor, Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL

Other Experience and Professional Memberships

2003-present Member, Biophysical Society
2010-present Member, American Heart Association
2005 Early Career Committee, Biophysical Society
2012-2013 Panelist, Early Career Development Committee, Biophysical Society
2014 Reviewer, NIGMS Program Projects Grants (P01) special emphasis panel
2015-present Member, Society for General Physiology
2015-2017 Reviewer, American Heart Association (Basic Cell, Proteins & Crystallography1 and Proteins & Crystallography 1 and 3)
2015-2021 Committee for Professional Opportunities for Women Committee (CPOW), Biophysical Society
2015-2018 Councilor (elected to office), Society for General Physiologists.
2016 Ad hoc Reviewer, NIH BBM study Section (Feb and Sep cycles).
2018 Ad hoc Reviewer, NIH BPNS study Section (Feb cycle).
2017 Reviewer, MCMB grant proposal, Medical Research Council (MRC) UK
2018 Editorial Advisory Board, Journal of General Physiology
2018 Reviewer, United States-Israel Binational Science Foundation
2018 Reviewer, French National Research Agency (ANR), France
2018 Reviewer, MCMB grant proposal, Medical Research Council (MRC) UK
2018-2020 Reviewer, United States-Israel Binational Science Foundation
2019 Editorial Board, Biophysical Journal
2019-2023 *Permanent Member*, Biochemistry and Biophysics of Membranes, NIH Study Section.

Honors

1995-1997 National Chemical Laboratory Scholarship, Pune, India
1997 Selected for Junior Research Fellowship, Council for Scientific and Industrial Research, India
1997-1999 Biomedical Engineering Scholarship, Indian Institute of Technology, Bombay, India
1999 Selected for the Cambridge Commonwealth Trust Scholarship and Overseas Research Scholar Award.
2004 Herbert Schuel Award for outstanding research in the field of Cell and Developmental Biology, University at Buffalo, SUNY.
2004 Dean's Award for Outstanding Dissertation, First Prize. University at Buffalo, SUNY.
2004 University at Buffalo nominee for the **CGS/UMI Distinguished Dissertation award.**
2005-2008 Postdoctoral Fellowship, American Heart Association
2007-2008 Postdoctoral Fellowship (Competitive Renewal), American Heart Association
2012-2016 Scientist Development Grant, American Heart Association.
2018 CWRU nominee for the Mallinckrodt Scholar Program.
2019-present Joseph T. Wearn, MD, University Professorship in Medicine

C. Contributions to Science

1. Structure-function relationships in nicotinic Acetylcholine receptors. One of the fundamental challenges in the ion channel field is to understand how spatially-separated structural motifs of the channel communicate in order to fine-tune its function. In my doctoral research, I addressed this question in nicotinic acetylcholine receptor-channels (nAChR) that belong to the neurotransmitter gated Cys-loop receptor family. These

channels are responsible for mediating fast synaptic transmission in neuronal and neuronal muscular junctions. Through single-channel current measurements of over 100 mutations and extensive model-based kinetic analysis within the framework of linear free energy relationships, I found that signal transduction occurs as a sequential movement of rigid "blocks" or "micro-domain" originating at the extracellular ligand-binding domain and culminating at the gate within the transmembrane region. Such an organized and linked motion of rigid bodies may underlie fast dynamics of the allosteric conformational change in these channels. This system also proved ideal to probe the speed-limits of global protein motions in the membrane. This finding has implications on barrier-less transitions in large multimeric membrane proteins.

a. **Chakrapani, S**., T.D. Bailey, and A. Auerbach. (2003). The role of loop 5 in acetylcholine receptor channel gating. ***J Gen Physiol***. 122:521-539. PMCID:PMC2229574
b. **Chakrapani, S**., T.D. Bailey, and A. Auerbach. (2004). Gating Dynamics of the Acetylcholine Receptor Extracellular Domain. ***J Gen Physiol***. 123: 341-356. (Featured on the Cover). PMCID:PMC2217457
c. **Chakrapani, S**., and A. Auerbach. (2005). A speed limit for conformational change of an allosteric membrane protein*.* ***Proc Natl Acad Sci U S A***, 2005. 102(1): p. 87-92. PMCID:PMC544059

2. C-type inactivation and modal gating behavior in K^+ channels. Studying prokaryotic channels provides a unique advantage to draw direct information from structural, dynamics, and functional measurements. However, unlike eukaryotic channels most of the bacterial members were not well-characterized at the functional level, this was particularly the case for KcsA, a pH-activated K^+ channel. As a part of my postdoctoral training, I carried out extensive kinetic analysis both at the macroscopic and single-channel level to characterize C-type inactivation and fast gating events that underlie KcsA function. To obtain high resolution structure of KcsA in multiple conformational states, I crystallized the channel in various mutant forms and in the presence of several modulators. Equating functional states to structural snapshots from crystallography, have led to a better understanding of the structural basis for inactivation from pre-open states, interaction of ions with the channel, modal gating behavior, and transitions that lead to fast gating events.

a. **Chakrapani, S**., Cordero-Morales, J. F., and Perozo, E. (2007a). A quantitative description of KcsA gating I: macroscopic currents. ***J Gen Physiol*** *130*, 465-478. PMCID:PMC2151670
b. **Chakrapani, S**., Cordero-Morales, J. F., and Perozo, E. (2007b). A quantitative description of KcsA gating II: single-channel currents. ***J Gen Physiol*** *130*, 479-496. PMCID:PMC2151667
c. **Chakrapani, S[a].**, Cordero-Morales, J. F[a]., Jogini, V., Pan, A. C., Cortes, D. M., Roux, R., and Perozo, E. (2011) On the structural basis for modal gating in K^+ channels ***Nature Structure & Molecular Biology*** *18 (1),* PMCID:PMC3059741. *[a]equal contribution.*
d. Ostmeyer J, **Chakrapani S**, Pan AC, Perozo E, Roux B. (2013) Recovery from slow inactivation in K+ channels is controlled by water molecules. ***Nature***. 501(7465):121-4. PubMed PMID: 23892782; PMCID:PMC3799803

3. Voltage-sensing mechanism and slow-inactivation in ion channels. Voltage-gated channels play a critical role in cellular excitability and thereby form the basis for initiation and propagation of nerve impulses. The structure of the voltage-sensor and the mechanisms underlying gating-charge movement have been areas intensively studied. Both the structure and the protein motions in the sensor are critically governed by the local membrane environment. Also as a part of my postdoctoral training, I used site-directed spin labeling and electron paramagnetic resonance (EPR) spectroscopy to directly investigate the architecture of the sensor in a reconstituted system. I studied the dynamics of the isolated voltage-sensors of prokaryotic K^+ (KvAP) and Na^+ (NaChBac) channels by EPR spectroscopy. These findings provided an in-depth view of the architecture of this domain on the membrane along with insights into the open-inactivated state of the channel. More recently, my lab characterized the molecular motions underlying slow-inactivation in voltage-gated Na+ channel (NavSp1) by pulsed-EPR spectroscopy.

a. **Chakrapani, S**., Cuello, L.G., Cortes, D.M., and Perozo, E. (2008). Structural dynamics of an isolated-voltage sensor domain in lipid bilayer. ***Structure*** *16, 398-409* PMCID:PMC2703488
b. **Chakrapani, S**., Sompornpisut, P., Intharathep, P., Roux, B. & Perozo, E. (2010). The activated state of a sodium channel voltage sensor in a membrane environment. ***Proc Natl Acad Sci U S A*** 107, 5435-40. PMCID:PMC2851821
c. **Chakrapani, S**. (2015) EPR studies of gating mechanisms in ion channels ***Methods in Enzymology*** 557:279-306 PMCID:PMC4503332
d. Chatterjee S, Vyas R, Chalamalasetti SV, Sahu ID, Clatot J, Wan X, Lorigan GA, Deschenes I, **Chakrapani S***. The voltage-gated sodium channel pore exhibits conformational flexibility during slow

inactivation. ***J Gen Physiol***. 2018;150(9):1333-47. doi: 10.1085/jgp.201812118. PubMed PMID: 30082431; PMCID: PMC6122925.
*This article was featured in a commentary "Progress in Understanding Slow Inactivation Speeds up" Payandeh, J ***Journal of General Physiology*** (2018)

4. Gating mechanisms in pentameric ligand-gated ion channels. Since joining the faculty at Case Western Reserve University as an Assistant professor in 2010, a major research focus of my lab has been to understand allosteric mechanisms in pentameric ligand-gated ion channels (pLGIC). Using prokaryotic homologues GLIC and ELIC as model systems, we elucidated the ligand-induced pore opening mechanism by EPR spectroscopy. Patch-clamp measurements from reconstituted channels were used to show the salient features of desensitization in GLIC that bears resemblance to the mechanism observed in the eukaryotic counterpart. These methods have allowed us to directly measure the effect of membrane lipid constituents on channel function and to determine the underlying changes in protein dynamics under these conditions. In addition, we studied long-range allosteric communications by engineering functional chimeric channels that incorporates domains from different members of the family. By using X-ray crystallography and pulse-EPR measurement, we determined the crystal structure of the chimera and measured ligand-induced structural changes which reveal conformational coupling between domains. More recently, my lab is geared towards applying these approaches in combination with cryo-EM to complex eukaryotic pLGIC. We recently determined the structures of the full-length 5-HT_{3A}R in the apo, and serotonin-bound conformations by single-particle cryo-EM. The structure reveals salient features of the resting, state and the conformational changes underlying serotonin-mediated activation. I served as the principal investigator in all these studies.

a. Basak, S., Gicheru, Y., Samanta, A., Molugu, S. k., Huang, W.,, de la Fuente, M., Hughes, T., Taylor, D.J., Nieman, M. T., Moiseenkova-Bell, V., and **Chakrapani, S*** (2018) Cryo-EM structure of 5-HT_{3A} receptor in its resting conformation. ***Nature Communications*** 9(1):514. doi: 10.1038/s41467-018-02997-4. PubMed PMID: 29410406. PMCID:PMC5802770
b. Basak S, Gicheru Y, Rao S, Sansom MSP, **Chakrapani S***. Cryo-EM reveals two distinct serotonin-bound conformations of full-length 5-HT3A receptor. ***Nature***. 2018;563(7730):270-4. doi: 10.1038/s41586-018-0660-7. PubMed PMID: 30401837. PMCID:PMC6237196 (*Article Recommended by Faculty 1000)*
c. Basak S[a], Gicheru Y[a], Kapoor A., Mayer ML., Filizola M, and **Chakrapani S***. (2019) Molecular mechanism of setron-mediated inhibition of full-length 5-HT3A receptors. ***Nature Communications*** 10, 3225, doi:10.1038/s41467-019-11142-8. PMCID:PMC6642186
d. Kumar A, Basak S, Rao S, Gicheru Y, Mayer ML, Sansom MSP, **Chakrapani S*.** (2020) Mechanisms of activation and desensitization of full length glycine receptors in lipid nanodisc. ***Nature Communications*** Jul 27;11(1):3752. doi: 10.1038/s41467-020-17364-5.PMID: 32719334

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/sudha.chakrapani.1/bibliography/50561146/public/?sort=date&direction=ascending

NAME: Kindig, Kayla Jeanne

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

POSITION TITLE: Ph.D Candidate

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
Case Western Reserve University, Cleveland OH	B.A.	05/2016	Biology
Case Western Reserve University, Cleveland OH	M.S.	05/2019	Biology
Case Western Reserve University, Cleveland OH	Ph.D.	05/2024 (Expected)	Physiology and Biophysics

A. Personal Statement

While I have always had an interest in science, my interest in research began in earnest when I was an undergraduate student. As a senior, I had to pick a topic for a final, in-depth research project. Since I was a child, I had suffered from chronic pain in the form of daily headaches, which range in severity from mild to debilitating and do not respond well to any medication. This is the topic that I chose for my capstone research review, and I would consider that my first foray into the world of neuroscience. I applied for a Master's program with the express intent of doing neurobiological research, as well as taking and teaching classes on neuroscience. I used electrophysiology to probe the function of cells responsible for converting auditory or vestibular stimuli into neural impulses, and this established my deep interest in electrophysiology and ion channels, along with the neurobiology lab I was teaching in which we run simulated electrophysiological experiments to illustrate concepts such as synaptic plasticity and cable theory. I became interested in structural biology when trying to find information on the structure of proteins we studied functionally in my Master's research and realizing there was surprisingly little information. I chose a PhD lab that was doing both structural and electrophysiological work, so that I could learn something new while also maintaining a connection with one of my passions. I am currently trying to solve the structure of a ligand-gated ion channel that acts under physiological conditions to inhibit nociceptive neurons and reduce the sensation of pain. Through a greater understanding of this protein, we can potentially design better analgesic drugs and help chronic pain sufferers such as myself without using medications that cause severe side effects due to nonspecific interactions with proteins that function elsewhere in the body.

B. Positions, Scientific Appointments, and Honors

2021-Present PhD Candidate, Department of Physiology and Biophysics, CWRU, Cleveland, OH
2020 Recknagel Academic Honors Award, CWRU, Cleveland, OH
2020 Recknagel Best Presentation Award, CWRU, Cleveland, OH
2017-2018 Teaching Assistant for Neurobiology Laboratory, CWRU, Cleveland, OH
2017-2018 Mentor in Cleveland Neuroscience Innovators Program, Cleveland, OH
2016-2018 Teaching Assistant for Intro Development and Physiology Lab, CWRU, Cleveland, OH
2018 Michelson Morley Competition Judge
2016 Member of Phi Beta Kappa

C. Contributions to Science

1. **M.S. Research:** During my Master's degree, my research was centered on understanding the function of vestibular and auditory hair cells both under physiological and pathophysiological conditions. One project that I worked on was focused on a particular family of mechanically-gated ion channels called TMCs

(transmembrane channel-like proteins), which had previously been implicated in cases of hereditary deafness. Our lab generated mutations in the genes encoding different TMC proteins, and I did *in vivo* electrophysiological recordings to assess the ability of the mutant hair cells to transmit mechanical signals into electrical impulses. We conducted these studies both in hair cells of the zebrafish lateral line that detect water flow and hair cells of the ear that detect auditory stimuli. We discovered a differential requirement for TMC proteins depending on hair cell type, providing us with information as to what proteins may be best suited to particular types of mechanical stimuli, which allows us to better understand the process of hearing and how it has evolved. A second project I developed and worked on was the investigation of protein composition and electrophysiological function between two different subpopulations of hair cells in the lateral line of zebrafish. A manuscript describing the findings of this research is currently in review, but in brief we found that two distinct types of hair cell that are polarized to optimally detect mechanical stimuli from two different directions actually have different intrinsic sensitivity to stimuli; this starts at the level of the mechanically gated channels, as shown by electrophysiological data and mutation of a candidate channel gene, and is conferred to the afferent neuron, which is shown by calcium imaging. These findings could be potentially translated into the study of human vestibular function in the maculae of the inner ear, where orthologous proteins are utilized to a similar effect.

- Chou S., Chen Z., Zhu S., Davis R.W., Hu J., Liu L., **Kindig K.**, Brown W., Fernando C.A., Stepanyan R., and McDermott B.M. Jr. (2017) A molecular basis for water motion detection by the mechanosensory lateral line. *Nature Communications* 8(2234).
- Chen Z.*, Zhu S.*, **Kindig K.***, Wang S., Chou S-W., Davis R.W., Dercoli M.R., Weaver H., Stepanyan R., and McDermott B.M. Jr. (2020) Tmc proteins are essential for zebrafish hearing where Tmc1 is not obligatory. *Human Molecular Genetics*, 29(12): 2004–2021. *Authors contributed equally

2. **Ph.D. Research:** My current research involves determination of the structure and function of glycine receptors, inhibitory ionotropic neurotransmitter receptors whose role is primarily in the spinal cord. I have worked on two different forms of homomeric glycine receptors, one involved in motor control and the other involved in perception of pain. I am using cryo electron microscopy (cryoEM) to determine the structure of these channels, in order to better understand the motions they go through during activation and to potentially help design better therapeutic drugs, with more receptor-specific binding properties and thus fewer off-target effects. I am also using electrophysiological techniques to connect the function of these receptors with the structure and examine the effect of mutations. Currently, we have a manuscript in review in which I performed all of the electrophysiology and generated all of the mutants used for experiments testing positive allosteric modulation. We used insights gained by the cryoEM structure to determine which residues to target for mutagenesis, and the resulting functional data provides us with a better understanding of the role of these residues in modulator binding. This protein is known to be mutated and have reduced functionality or expression in cases of hyperekplexia, a motor disease characterized by an excessive startle response and muscle rigidity. Positive allosteric modulators of this protein can be used to ameliorate the effects of this disease, and structural insight is crucial to design drugs that are highly specific and potent.