BIOGRAPHICAL SKETCH

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NAME: Chakrapani, Sudha

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

POSITION TITLE: Associate 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. An area of emphasis of my research is in understanding the critical interaction between ion channels and membrane lipids, and in determining how this interaction is altered in the presence of allosteric modulators such as neurosteroids, alcohols, and anesthetics. My scientific approach is a combination of cutting-edge multidisciplinary tools that includes electrophysiology (patch-clamp measurements in HEK-293 cells and reconstituted proteoliposomes; two-electrode voltage-clamp measurements in oocytes), spectroscopy (EPR), X-ray crystallography, and more recently single-particle cryo-EM. During my doctoral research, I was trained in nAChR single-channel recordings and model-based kinetic analysis. As a part of my postdoctoral training, I gained expertise in membrane protein biochemistry, EPR spectroscopy, and X-ray crystallography. My lab was trained in single-particle cryo-EM in collaboration with Dr. Vera Moiseenkova-Bell, and these efforts were facilitated by a two-year NIH “Cryo-Electron Microscopy Technology Transfer” administrative supplement to my R01. We solved the cryo-EM structure of the full-length 5-HT_{3A}R channel in its resting conformation (*Nature Communications*). My lab recently determined the structures of 5-HT_{3A}R in serotonin-bound states that revealed the conformational changes underlying channel activation (*Nature*). 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). All aspects of sample preparation, grid optimization, data processing, and structure determination for this work were carried out by my group.

1. 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. (*Article Recommended by Faculty 1000)*
2. 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.
3. Basak, S. [a], Schmandt, N. [a], Gicheru, Y [a]., and **Chakrapani, S*.** (2017) Crystal Structure and dynamics of a Lipid-induced Potential Desensitized State of a Pentameric Ligand-gated Channel ***eLIFE,*** doi: 10.7554/eLife.23886.
4. Schmandt, N., Velisetty, P., Chalamalasetti, S. V., Stein, R. A., Bonner, R., Talley, L., Parker, M. D., Mchaourab, H. S., Yee, V. C., Lodowski, D. T., and **Chakrapani, S*.** (2015) An ELIC-GLIC Chimera Reveals Distinct Pathways of Activation in the Cys-loop receptors. **J Gen Physiol**, Oct; 146(4):323-40. PMCID:PMC4586589

B. Positions and Honors.

Positions and Employment

2008-2010 Research Assistant Professor, Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL
2010-2017 Assistant Professor (Tenure-track), Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH
2017-present Associate Professor (Tenured), Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH
2018-present Director, Cryo-Electron Microscopy Core, Case Western Reserve University, Cleveland, OH

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-present 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
2019 Editorial Board, Biophysical Journal

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.

C. Contribution 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.

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. Schmandt, N., Velisetty, P., Chalamalasetti, S. V., Stein, R. A., Bonner, R., Talley, L., Parker, M. D., Mchaourab, H. S., Yee, V. C., Lodowski, D. T., and **Chakrapani, S.** (2015) An ELIC-GLIC Chimera Reveals Distinct Pathways of Activation in the Cys-loop receptors. **J Gen Physiol**, Oct;146(4):323-40. PMCID:PMC4586589
b. Basak, S. [a], Schmandt, N. [a], Gicheru, Y [a]., and **Chakrapani, S*.** (2017) Crystal Structure and dynamics of a Lipid-induced Potential Desensitized State of a Pentameric Ligand-gated Channel (***eLIFE,*** doi: 10.7554/eLife.23886).
c. 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.
d. 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. (*Article Recommended by Faculty 1000)*

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

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

NIH R01 GM131216 **Chakrapani (PI)** 01/1/19 – 21/31/22
Title: "Structure, Function, and Modulation of Serotonin (3A) receptors"
The goal of this award is to elucidate the structural changes associated with gating and modulation in full-length serotonin-3A receptors.

NIH R01 GM108921 **Chakrapani (PI)** 09/1/14 – 08/31/19
Title: "Molecular Mechanisms of Desensitization and Drug Modulation in Ligand-Gated Ion Channels."
The goal of this award is to determine the structural basis for desensitization in ligand-gated channels and how transitions to this state are regulated by endogenous and exogenous modulators.

NIH R01 GM108921-5S1 **Chakrapani (PI)** 09/1/18 – 08/31/19
Molecular Mechanisms of Desensitization and Drug Modulation in Ligand-Gated Ion Channels.
Administrative Supplement for small equipment Role: PI

NIH S10 OD025259 **Chakrapani (PI)** 09/01/18 – 08/31/2019
Title: "Pulsed-Electron Paramagnetic Resonance Spectrometer for Distance Determination in Biological Macromolecules"
The overall goal of this award is to establish the first pulsed-EPR capability in the Greater Cleveland Area.

Completed Research Support

NIH 3R01GM108921-03S1 **Chakrapani (PI)** 09/1/16 – 08/31/18
Title: "Molecular Mechanisms of Desensitization and Drug Modulation in Ligand-Gated Ion Channels"
Collaborative Supplements for Cryo-Electron Microscopy Technology Transfer (Admin Supp) to develop technical expertise in high resolution cryo-electron microscopic (cryoEM) single particle analysis in the PI's lab

NCRP Scientist Development Grant **Chakrapani (PI)** 12SDG12070069 07/01/12 – 06/30/16
American Heart Association
Title: "Structural dynamics of gating and selectivity in Voltage-gated sodium channels."
The overall goal of this award is to understand the conformational changes associated with voltage-dependent gating and selective ion permeation in voltage-gated channels.

NIH 1R01 GM099665-01 Maguire (PI) 09/24/12 – 05/31/14
Project Title: "Magnesium Channel Cation Selectivity". Role: Co-Investigator