BIOGRAPHICAL SKETCH

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NAME: Gouaux, James Eric

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

POSITION TITLE: Senior Scientist

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
Harvard College, Cambridge MA	AB	1984	Chemistry
Harvard University, Cambridge MA	PhD	1989	Physical chemistry
Harvard University, Cambridge MA	Postdoc	1989-90	Crystallography
Massachusetts Institute of Technology, Cambridge MA	Postdoc	1990-92	Membrane proteins

A. Personal Statement

My research focuses on the molecular mechanisms underpinning signal transduction at chemical synapses. To do this, I have primarily employed x-ray crystallographic methods to elucidate atomic resolution structures of crucial neurotransmitter receptors and transporters, yet I have also enthusiastically engaged and learned complimentary biochemical and biophysical methods with the ultimate aim of using all possible approaches to elaborate structure-based mechanisms. Thus, I have extensive experience in the expression, characterization and crystallization of complex neurotransmitter receptors and transporters, as well as in x-ray crystallography and electrophysiology. In addition, I have now established single particle cryo EM in my laboratory as a central method by which to elucidate neurotransmitter receptor structures. As evidence of my progress in this area, I have published multiple papers in which we have used single particle cryo-EM as the primary tool to elucidate molecular structure and, together with biochemical, electrophysiological and computational approaches have gone on to define structure-based mechanisms for important receptors and transporters. I also participate in leadership of the PNCC, an NIH-funded, national cryo-EM center.

Projects to highlight include:

NIH 2 R01 NS038631-24
Gouaux, James Eric (PI)
03/19/1999-02/28/2025
Structure and Function of Neurotransmitter Transporters

NIH 5 R01 MH070039-19
Gouaux, James Eric (PI)
07/01/2004-02/29/2024
Structure and Function of Neurotransmitter Transporters

NIH 5 R01 GM100400-08
Gouaux, James Eric (PI)
06/01/2012-03/31/2022
Structural biology of neurotransmitter ion channels

HHMI (no number)
Gouaux, James Eric (PI)
09/01/2010-08/31/2027
Molecular Studies of Synapses

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2015-Present	Jennifer and Bernard Lacroute Term Chair in Neuroscience Research, Portland OR
2005-Present	Senior scientist, Vollum Institute, Oregon Health and Science Univ., Portland OR
2000- Present	Investigator, Howard Hughes Medical Institute
2001-2005	Professor, Dept. Biochem. Mol. Biophys., Columbia Univ., New York NY
2000-2001	Associate professor, Dept. Biochem. Mol. Biophys., Columbia Univ., New York, NY
1996-2000	Assistant professor, Dept. Biochem. Mol. Biophys., Columbia Univ., New York NY
1993-1996	Assistant professor, Dept. Biochem. Mol. Biol., Univ. Chicago, Chicago IL

Honors

2020	National Academy of Medicine Member
2016	Anatrace Membrane Protein Award, Biophysical Society
2014	Honorary Doctorate, University of Copenhagen
2014	W. Alden Spencer Award, Columbia University
2014	Alexander M. Cruickshank Lecture, Gordon Research Conferences
2013	Physiological Society Annual Review Prize Lecture
2010	Distinguished Faculty Awards Winner for Outstanding Research
2010	National Academy of Sciences Member
2009	Medical Research Foundation Discovery Award, Oregon Health & Science University
2009	NIHMH MERIT Award
2008	NINDS Javits Investigator Award
2007	American Association for the Advancement of Science Fellow
2003	P&S Dean's Distinguished Award in the Basic Sciences, Columbia University
2000	P&S Doctor Harold & Golden Lamport Award for Excellence in Basic Science Research, Columbia University
1998	Klingenstein Research Fellow
1997	Alfred P. Sloan Research Fellow
1995	National Science Foundation Young Investigator
1994	Searle Scholar

C. Contributions to Science

My major contributions have been to provide a molecular basis for understanding the function of neurotransmitter receptor and transporters, fundamental molecular machines that mediate signal transduction at the chemical synapses of the central nervous system. We have focused on ionotropic glutamate receptors, acid sensing ion channels, ATP-gated P2X receptors and pentameric Cys-loop receptors, as well as on the transporters for glutamate and the biogenic amines. My work has not only provided insights into the three-dimensional structures of these crucial receptors and transporters, but because all of our results are deposited in the publicly accessible protein data bank, the results of my work are available to everyone throughout the world. Thus, our studies will not only inform society on the fundamental building blocks of the brain, but they will also provide a foundation for those who are devoted to developing new therapeutic agents.

1. Our studies on the ionotropic glutamate receptors have provided deep insight into their mechanism of action, showing how antagonists, agonists and allosteric modulators act on these fundamental receptors.

a. Zhao Y, Chen S, Swensen AC, Qian WJ, Gouaux E. Architecture and subunit arrangement of native AMPA receptors illuminated by cryo-EM. Science 364, 355-362 (2019). PMCID: PMC6701862
b. Zhu S. Stein RA, Yoshioka C, Lee CH, Goehring A, Mchaourab HS, Gouaux E. Mechanism of NMDA receptor inhibition and activation. Cell 165: 704-14 (2016). PMCID: PMC4914038
c. Chen S, Zhao Y, Wang Y, Shekhar M, Tajkhorshid E, Gouaux E. Activation and desensitization mechanism of AMPA receptor-TARP complex by cryo-EM. Cell 170:1234-1246 (2017). PMCID: PMC5621841

2. We have also elaborated the molecular structure of the two major classes of neurotransmitter transporters, showing how these remarkably machines carry neurotransmitter from one side of the membrane to the other.

a. Coleman JA, Yang, D, Zhao, Z, Wen, PC, Yoshioka, C, Tajkhorshid, E, Gouaux, E. Serotonin transporter ibogaine complexes illuminate mechanisms of inhibition and transport. Nature 569, 141-145 (2019). PMCID: PMC6750207
b. Coleman JA, Green EM, Gouaux E. X-ray structures and mechanism of the human serotonin transporter. Nature 532: 334-39 (2016). PMCID: PMC4898786
c. Wang KH, Penmatsa A, Gouaux E. Neurotransmitter and psychostimulant recognition by the dopamine transporter. Nature 521:322-27 (2015). PMCID: PMC4469479

3. In addition, we have elaborated the structures of other neurotransmitter receptors and ligand gated ion channels of the brain, from acid sensing ion channels and ATP-gated P2X receptors to pentameric Cys-loop receptors, thus providing the neuroscience field with molecular blueprints upon which to ground studies of mechanism and drug development.

a. Du J, Lü W, Wu S, Cheng Y, Gouaux E. Glycine receptor mechanism illuminated by electron cryo-microscopy. Nature 526:224-29 (2015). PMCID: PMC4659708
b. Baconguis I, Bohlen, CJ, Goehring A, Julius D, Gouaux E. X-ray structure of acid-sensing ion channel 1–snake toxin complex reveals open state of a sodium-selective channel. Cell 156:717-29 (2014). PMCID: PMC4190031
c. Mansoor SE, Lü W, Oosterheert W, Shekhar M, Tajkhorshid E, Gouaux E. X-ray structures define human P2X3 receptor gating cycle and antagonist action. Nature 538: 66-71 (2016). PMCID: PMC5161641.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/james.gouaux.1/bibliography/40629156/public/?sort=date&direction=ascending

BIOGRAPHICAL SKETCH

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NAME: Freitas, Makayla

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

POSITION TITLE: Graduate Student

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	START DATE MM/YYYY	END DATE MM/YYYY	FIELD OF STUDY
University of Arizona, Tucson, AZ	N/A	08/2014	01/2017	Nursing
University of Arizona, Tucson, AZ	BS	01/2017	05/2019	Mol. Cellular Biology; Psychology
Oregon Health & Science University, Portland, OR	PhD	09/2019	09/2024	Neuroscience

A. Personal Statement

The goal of my proposed research is to combine atomic-level structural studies and functional electrophysiology assays to determine the resting state structure, molecular gating mechanism, and synaptic regulation of an acid-sensing ion channel. An eclectic educational background and extensive multidisciplinary research experience in relevant scientific disciplines make me an ideal candidate to serve as principal investigator for the research I am proposing. My undergraduate research utilized structural biology, biophysics, and biochemistry techniques to study signal transduction under the mentorship of Dr. Rebecca Page. My first project utilized surface plasmon resonance (SPR), site-directed mutagenesis, and X-ray crystallography to study how Calcineurin (CN) interacts with its substrates through short linear interaction motifs (SLIMs). My second project aimed to investigate the molecular mechanism that underlies the activation of nuanced Ser/Thr Protein Phosphatase 1 (PP1). Under the combined mentorship of Dr. Rebecca Page and Dr. Wolfgang Peti, I incorporated protein nuclear magnetic resonance (NMR) to determine the interactions and dynamics between PP1 and its target proteins.

I chose to pursue my doctorate in neuroscience at the Vollum Institute at Oregon Health and Science University, an electrophysiology power house, and joined the lab of Dr. Eric Gouaux, a world-renowned leader in membrane protein structural biology. My proposed research builds upon my past experience in structural biology, while also broadening my biochemistry skillset to include membrane proteins and expanding my biophysical toolkit to include cryo-EM and electrophysiology. In summary, my independent research experience in a relevant discipline, as well as a strong educational background demonstrates my capacity to successfully lead the research I am proposing.

B. Positions and Honors

Positions and Employment

Activity/ Occupation	Start Date (mm/yy)	End Date (mm/yy)	Field	Institution	Supervisor/ Employer
General Chemistry TA	08/2015	12/2016	Chemistry	University of Arizona	Laura Van Dorn, PhD
Supplemental Instruction (S.I.) Leader	01/2017	02/2018	Chemistry	University of Arizona	Think Tank
Undergraduate Research Assistant	05/2017	05/2019	Structural Biology	University of Arizona	Rebecca Page, PhD

Chemistry Independent Study	08/2017	01/2018	Chemistry Education	University of Arizona	Vicente Talanquer, PhD
Research Assistant	05/2019	07/2019	Structural Biology	University of Arizona	Rebecca Page, PhD; Wolfgang Peti, PhD
Rotation Student	07/2019	09/2019	Structural Biology/ Neuroscience	Oregon Health and Science University	Eric Gouaux, PhD
Rotation Student	12/2019	02/2020	Neurophysiology	Oregon Health and Science University	John T Williams, PhD
Rotation Student	02/2020	04/2020	Neurophysiology	Oregon Health and Science University	Swetha Murthy, PhD
Graduate Student	04/2020		Structural Biology/ Neuroscience	Oregon Health and Science University	Eric Gouaux, PhD

Academic Awards

2018 – Excellence in Biological Science Research Scholar, University of Arizona
2018 – WAESO Scholarship, National Science Foundation (NSF)
2018 – Galileo Circle Scholar, The Galileo Circle
2019 – WAESO Scholarship, National Science Foundation (NSF)
2019 – Galileo Circle Scholar, The Galileo Circle
2019 – Promising Graduate Student Scholar, Oregon Health Science University
2019 – Achievement Rewards for College Scientist award, ARCS Foundation (3-year funding)
2020 – Gordon Research Conference Carl Storm Underrepresented Minority (CSUM) Fellowship
2020 -- Neuroscience Scholar Program Associate, Society for Neuroscience (SFN) (2-year program)

Grants

2018 – Undergraduate Biology Research Program (UBRP) Fellowship, University of Arizona (1-year funding)
2020 – Pacific Northwest Center for Cryo-EM (PNCC), 120 hours of access to microscopes.
2020 – Ruth L Kirschstein's National Research Service Award (NRSA, F31NS120713); 2020-2024
2021 – National Science Foundation Graduate Research Fellowship (NSFGRF)

C. Contribution to Science

My most significant contribution to science to date is centered upon expanding the current understanding of how signals from outside the cells are transmitted to the nucleus. To do this, I have used structural biology, biophysics, neurophysiology and biochemistry to study how highly dynamic proteins rapidly and precisely transmit messages at atomic resolution. While working at the University of Arizona in the laboratory of Dr. Rebecca Page and Dr. Wolfgang Peti, I studied how targeting proteins direct the function of ser/thr phosphatase, specifically Protein Phosphatase 2B (Calcineurin) and Protein Phosphate 1 (PP1). Currently, as a graduate student at Oregon Health Science University, in the laboratory of Dr. Eric Gouaux, I am studying the architecture and mechanism of acid gated ion channels and how they augment chemical synapses of the nervous system.

1. Identifying mechanism underlying Calcineurin: AKAP79: RIIα

Calcineurin (CN) function is regulated and directed by target proteins that carry two distinct short linear interaction motifs (SLiMs), the PxIxIT and πΦ-LxVP motif. It was previously thought that all target proteins of CN carry both CN recognition motifs independently until two known CN target proteins, A-kinase anchoring protein 79 (AKAP79) and the regulatory domain of PKA (RIIα), were found to each only contain a single CN recognition motif: A-kinase AKAP79 containing the PxIxIT, and RIIα containing only the πΦ-LxVP motif. I used surface plasmon resonance and site-directed mutagenesis to determine if, and at what affinities, two independent substrates, A-kinase AKAP79 and RIIα, could independently interact with CN to form a signaling complex. My

data showed that these two independent substrates do bind in tandem to CN, and to each other, with nanomolar affinity, and that the recently identified non- canonical LxVP (LKIP) found on AKAP79 does interact with CN with micromolar affinity, increasing the affinity of AKAP79 to CN to nanomolar affinity. Co-crystallization of CN bound to truncated AKAP79 and RIIα were solved, further confirming these results. My work elucidated mechanistic details of the first identified system that binds CN as a triple complex and that does so with nanomolar affinity.

1. **Freitas MM**, Moon TM, Peti W, Page R. Biological, Engineering, and Chemical Undergraduate Research (BECUR) Conference, Tucson, Arizona, March 2018.
2. **Freitas MM**, Moon TM, Peti W, Page R. 30th Annual Undergraduate Biology Research Program Conference, Tucson, Arizona, January 2019.
3. Moon TM, **Freitas MM**, Page R, Peti W. (2020). Calcineurin-specific SLiMs encoded on distinct proteins combine to form a high-affinity complex with Calcineurin. (Manuscript completed, waiting on collaboration.)

2. Investigating the role of p37 in the activation of Protein Phosphatase 1

During the biogenesis of PP1, the nuanced PP1 forms a heterotrimetic complex, Inhibitor3:PP1:SDS22, which stabilizes PP1 in a resting state. As long as PP1 is bound between SDS22 and I3, PP1 remains inactive and is unable to dephosphorylate its substrates. The crucial mechanism of how PP1 is released from the tight complex remains unknown. A study from the Bollen Lab implicated p37, a cofactor of the AAA-type A ATPase, as coordinating PP1's release from the complex. Using protein NMR, I set out to investigate what p37's role was in the dissociation of Inhibitor-3 from the I3:PP1:SDS22 complex. Although I completed my degree before this project was finished, I independently completed and analyzed titration studies of 15Np37:I3, 15Np37:I3/PP1, 15Np37:PP1, and 15Np37:SDS22 and assigned the secondary structure of Δ82 p37 (30 kD protein) to 96%.

1. **Freitas MM**, Peti W, Page R. Biological, Engineering, and Chemical Undergraduate Research (BECUR) Conference, Tucson, Arizona, March 2019.
2. **Freitas MM,** Peti W, Page R. UA's Chemistry & Biochemistry Senior Thesis Conference, Tucson, Arizona, April 2019.
3. **Freitas MM**, Peti W, Page R. (2019) Uncracking p37's role in the activation of Protein Phosphatase 1, *Published, Senior Thesis*

3. Determining the molecular architecture and mechanism of acid-sensing ion channel 5

The aims of this project are to elucidate the molecular architecture and mechanism of ASIC5 channel. Since developing my research plan, I have developed optimized methods to recombinantly express and purify ASIC5 in its oligomeric state and reconstitute the channel into a nanodisc. Results from this study will translate into a greater understanding of the structure/function relationship of this channel.

1. **Freitas MM**, Gouaux E. The molecular mechanism and architecture of ASIC5. Talk given at Work-in-progress student seminar: The Vollum Institute at Oregon Health Science University; Aug 2020