



Doctoral Dissertation Defense Announcement

“The Role of Sorting Nexin 9 Family in G Protein-Coupled Receptor Regulation”



Valeria L. Robleto Montealegre

Candidate for Doctor of Philosophy

Biochemistry

School of Graduate Studies

Medical College of Wisconsin

Committee in Charge:

Adriano Marchese, PhD (Mentor)

John Corbett, PhD

Amy Hudson, PhD

Jong-In Park, PhD

John Auchampach, PhD

Date: Wednesday, June 12th, 2024

Time: 11:00 AM (CST)

Defense Location: Kerrigan Auditorium

Zoom: <https://mcw-edu.zoom.us/j/4927134272?pwd=RC9EZFcYUWQ0OWk0Q2ovWGF6d08rZz09&omn=983184222>
95

Meeting ID: 492 713 4272 Passcode: cwSWt1VP

Graduate Studies:

Intro to Biomedical Research

Biochemistry of the Cell

Techniques in Molecular and Cell Biology

Molecular and Cellular Biology

Classical and Molecular Genetics

Mechanisms of Cellular Signaling

Ethics and Integrity in Science

Research Ethics Discussion Series

Protein Chemistry Applications

Protein Chemistry Principles

Biochemistry Seminar

Research and Reading

Doctoral Dissertation

Dissertation

“The Role of Sorting Nexin 9 Family in G Protein-Coupled Receptor Regulation”

Abstract

G protein-coupled receptors (GPCRs) sense extracellular stimuli and mediate almost every aspect of human physiology, including appropriate functioning of the cardiovascular, gastrointestinal, endocrine, nervous, and immune systems. Activating ligands promote coupling of GPCRs with heterotrimeric G-proteins that transduce extracellular signals into an intracellular signaling response. Dysregulation of this signaling is linked to many diseases, such as cancer, Alzheimer's, drug addiction, obesity, and autoimmune disorders. Despite the role of GPCRs in disease, the mechanisms that regulate GPCR signaling remain poorly understood. The overall goal of this project is to elucidate the mechanisms responsible for GPCR regulation. GPCR signaling is tightly regulated by GPCR kinases (GRKs) and multi-faceted adaptor proteins called β -arrestins. β -arrestins bind to ligand-activated and GRK-phosphorylated GPCRs at the plasma membrane. β -arrestin binding prevents G protein coupling while targeting GPCRs for internalization by their ability to simultaneously scaffold components of the internalization machinery. In addition, β -arrestins can activate various signaling pathways independent of G proteins. However, the mechanism by which β -arrestins perform these functions remains poorly understood, mainly because the full extent of their interacting partners remains unknown. To discover novel GPCR-stimulated β -arrestin1 interacting partners, we used peroxidase-based proximity labeling coupled with affinity purification and quantitative mass spectrometry by appending the ascorbic-based peroxidase APEX in-frame to the C-terminus of β -arrestin1. This does not disrupt the role of β -arrestin1 in GPCR internalization or its ability to bind to known interacting proteins. Using this approach several β -arrestin1-interacting proteins were found to be increased in abundance following GPCR stimulation. Biochemical experiments confirmed that two proteins interact with β -arrestin1, which we predict are novel ligand-stimulated β -arrestin1 interacting partners. One of these proteins is sorting nexin 9 (SNX9), an endocytic remodeling protein that has pleiotropic effects on endocytosis and signaling but has never been linked to GPCR regulation. Using the chemokine receptor CXCR4 as a model GPCR, we show by RNAi and using gene-edited SNX9 and β -arrestin knockout cells that SNX9, but not β -arrestins, is essential for agonist-stimulated CXCR4 internalization. We show in cells that SNX9 is recruited to CXCR4 at the plasma membrane in an agonist-dependent manner independent of the interaction with β -arrestin1. Further, we show that SNX9 binds directly to the cytoplasmic carboxyl-terminal tail of CXCR4 in a phosphorylation-dependent manner. Receptor mutants that are unable to recruit SNX9 are also unable to internalize. β -arrestins are also recruited to agonist-stimulated CXCR4, which is mutually exclusive with SNX9 recruitment. Furthermore, we provide evidence that some receptors do not require SNX9 nor β -arrestins for endocytosis, suggesting additional modes for GPCR endocytosis. We propose that SNX9 mediates CXCR4 internalization, while β -arrestins mediate receptor desensitization and G protein-independent signaling. Collectively, our data are consistent with a model in which SNX9 functions as a cargo adaptor that defines a novel mode of GPCR endocytosis. These results provide novel insights into the mechanisms regulating GPCR trafficking and broaden our overall understanding of GPCR regulation.

Valeria Robleto

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EDUCATION

- Doctor of Philosophy in Biochemistry (Expected: Summer 2024)** **2018 – present**
Medical College of Wisconsin (MCW) **Milwaukee, WI**
Department of Biochemistry
“Role of Sorting Nexins in GPCR Regulation”
- Bachelor of Science in Chemistry and Biology (Summa Cum Laude)** **2014 – 2018**
University of the Ozarks **Clarksville, AR**
Areas of concentration: Molecular and Cellular Biology

AWARDS AND HONORS

- MCW Graduate School Poster Session Award, December 2023
- ASPET (American Society of Pharmacology and Experimental Therapeutics) Travel Award, May 2023
- Graduate School Association Travel Award, May 2023
- Medical College of Wisconsin Graduate School Association Top Abstract Award, April 2023
- FASEB (Federation of American Societies for Experimental Biology) Travel Award, August 2022
- ASPET Washington Fellow, Spring 2021
- Minority Scholarship at MCW 2020
- Honor’s List (Dean’s/President’s List), yearly 2014-2018
- Tri-Beta South Central Regional Convention First Place in Oral Presentation Competition, 2018
- Student Success Center Chemistry Student of the Year, University of the Ozarks, 2018
- Student Success Center Political Science Tutor of the Year, University of the Ozarks, 2018
- Student Success Center Chemistry Tutor of the Year, University of the Ozarks, 2017
- Tri-Beta Research Grant, 2017
- INBRE (IDeA Network of Biomedical Research Excellence) Research Grant, 2017
- Member of Tri-Beta Biological Honor Society
- Student Government Association Senator of the Year, University of the Ozarks, 2015-2016
- United States Delegate for University Scholars Leadership Symposium, Hanoi Vietnam, 2016.
- Walton International Scholarship Program (full college tuition, housing, and allowance).

RESEARCH EXPERIENCE

Graduate Student Researcher

Medical College of Wisconsin (MCW)

Advisor: Adriano Marchese, Ph.D

2019 – present

Milwaukee, WI

- Validating a novel interaction between Sorting Nexin 9 (SNX9), CXCR4, and/or β -Arr1 using cell culture model systems coupled with several approaches, including biochemical, RNA interference, CRISPR/Cas9, BRET, and microscale thermophoresis techniques.
- Presenting scientific findings on the role of SNX9 in regulating GPCR signaling and endocytosis at scientific conferences through oral and poster communication.

Graduate Student Rotations

Medical College of Wisconsin (MCW)

Laboratory of Carmen Bergom, M.D., Ph.D.

August 2018–May 2019

Milwaukee, WI

- Characterized differences between T-cell infiltration and vascular density of heart tissue of SS (Sprague Dawley derived) and SSBN3(SS and Brown Norway inbred) consomic rats.

Laboratory of Marja Nevalainen, M.D., Ph.D.

- Prepared protein samples from prolactin-stimulated CWR22RV1 cells previously transfected with lentivirus for androgen receptor knockdown and tested for STAT5 phosphorylation via immunoblotting.

Laboratory of William Drobyski, M.D., Ph.D.

- Performed bone marrow transplants to BALB/c mice and subsequently harvested spleens and lymph nodes and studied CCR7 expression by flow cytometry.

Summer Undergraduate Research Fellowship (SMART)

Baylor College of Medicine

Laboratory of Debananda Pati, Ph.D.

May–July 2017

Houston, TX

- Cloned human Separase
- Developed expression technique for human Separase by co-transfecting with mutants of its chaperone protein Securin.

Undergraduate Student Research

University of the Ozarks

Laboratory of Befrika Murdianti, Ph.D.

August 2017–May 2018

Clarksville, AR

- Synthesized Graphene Oxide and tested its ability to filter metals out of water (Chromium and Cobalt) using atomic absorption spectrometry.

Laboratory of Sean T. Coleman, Ph.D.

- Collected soil samples from farms that raised cattle with or without antibiotic treatment and tested for the presence of bacteria with antibiotic resistance. Funded by INBRE and Tri- Beta grants.

PUBLICATIONS

- **Robleto VL**, Zhuo Y, Crecelius JM, Benzow S, Marchese A. SNX9 mediates β arrestin-independent GPCR internalization. *Under Review, Communications Biology*.

- Zhuo Y*, **Robleto VL***, Marchese A. Proximity Labeling to Identify β -Arrestin1 Binding Partners Downstream of Ligand-Activated G Protein-Coupled Receptors. *Int J Mol Sci*. **2023 Feb 7**;24(4):3285. doi: 10.3390/ijms24043285. PMID: 36834700; PMCID: PMC9967311. *Authors contributed equally to this work.

TEACHING

Student Facilitator

Medical College of Wisconsin (MCW)

Courses: Biochemistry

- Attended graduate-level biochemistry courses and helped students navigate and solve in-class assignments.
- Graded in-class assignments of graduate-level biochemistry.

Fall of 2022 and 2023

Milwaukee, WI

Student Coach

Medical College of Wisconsin (MCW)

Courses: Cell Biology and Cell Signaling

- Coached first-year graduate students and helped them prepare for exams in cell biology and cell signaling.

August–November 2020 & August–November 2022

Milwaukee, WI

Teaching Assistant

University of the Ozarks

Courses: Principles of Cell Biology (BIO2314) and Microbiology (BIO3134)

- Prepared the materials required for the laboratory section of the class.
- Assisted students during the laboratory section of the class
- Held a course tutorial once a week to answer questions about the class

January–May 2016

Clarksville, AR

Personal Tutor and Note Taker

University of the Ozarks, Student Success Center

Courses: General Chemistry I and II (CHM1014 and CHM1024), Cells and Developmental Biology (BIO1324), Organic Chemistry I and II (CHM3014 and CHM3024), Microbiology (BIO3134), Biochemistry (CHM4104), International Relations (PLS2033) and American National Government (PLS2013) as needed.

- Met with students as needed to tutor them on courses, provide them with study skills, and help them prepare for exams.

August 2016–May 2018

Clarksville, AR

Personal Tutor and Note Taker

University of the Ozarks, Jones Learning Center

Courses: Trigonometry (MTH1043), Calculus I (MTH2015), Introduction to Business (BSA1103) and Old Testament (REL1003).

- Met with students with learning disabilities as needed to tutor them on courses, provide them with study skills, and help them prepare for exams.

August 2016–May 2018

Clarksville, AR

MENTORSHIP

Katy LaFond, Graduate Rotation Student

2021

Graduate Student Mentor, Medical College of Wisconsin

Milwaukee, WI

- Trained student during 6-week period on tissue culture technique, and on cell migration assays to be set up in a confocal microscope.
- "B-cell chemotaxis in response to CXCL12 and CXCL13".

Miracle Emosibve, Graduate Rotation Student

2021

Graduate Student Mentor, Medical College of Wisconsin

Milwaukee, WI

- Developed a project and then trained the student during a 6-week period on tissue culture technique, cloning, and molecular-based techniques such as signaling assays and western blotting.
- "Role of Sorting Nexin 9 in CXCR4 Degradation".

FELLOWSHIPS AND RELEVANT EXPERIENCE

ASPET Washington Fellow

April 2021

Virtual

- Organized meetings with congressmen and congresswomen or their liaisons and met with congressmen and congresswomen or their liaisons to advocate for funding for the NIH and support for animal research.

Laboratory Assistant

June-July 2016

Cerveceria Nacional de Nicaragua (Nicaragua's Beer Company)

Managua, Nicaragua

Microbiology Quality Control Laboratory.

- Ran quality control assays on samples of purified water, juice, and beer utilizing bacterial and fungal culture, and filtration methods.
- Analyzed viability and vitality of yeast for fermentation and beer preparation.

Student Ambassador

August 2016-May 2018

University of the Ozarks, Admissions.

Clarksville, AR

- Represented the University of the Ozarks while giving tours of the campus and introducing potential students and their parents to professors and coaches.

INVITED TALKS

- ASPET 2023, St. Louis, MO. **Datablitz.**
 - Title: Regulation of GPCR Signaling by Sorting Nexins
 - Authors: Valeria Robleto, Ya Zhuo, Joseph Crecelius, Adriano Marchese
- Medical College of Wisconsin Graduate School Association, April 2023 (*Top Abstract Awards*)
 - Title: Role of Sorting Nexins on GPCR Regulation
 - Authors: Valeria Robleto, Ya Zhuo, Joseph Crecelius, Sara Benzow, Adriano Marchese
- Tri-Beta South Central Regional Convention 2018, Dallas, TX.
 - Title: Cloning and Expression of Human Separase. (*First Place*)
 - Authors: Valeria Robleto, Debananda Pati

INVITED TALKS (Continued)

- ARCH Symposium at University of the Ozarks 2017, Clarksville, AR.
 - Title: Internship at Nicaragua's Brewery Microbiology Quality Control Laboratory. (*Honorable Mention*)
 - Authors: Valeria Robleto

POSTERS AND ABSTRACTS

- Graduate School Poster Session 2022, Milwaukee, WI. (*Top Poster Award*)
 - Title: Regulation of GPCR Signaling by Sorting Nexins
- ASPET 2023, St. Louis, MO.
 - Title: Regulation of GPCR Signaling by Sorting Nexins (*Selected for poster competition*)
 - Authors: Valeria Robleto, Ya Zhuo, Joseph Crecelius, Adriano Marchese
- The G Protein-Coupled Receptor Kinases and Arrestin Conference: Key Modulators in Cells Signaling 2022, Jupiter, FL-FASEB.
 - Title: Proximity labeling to identify novel β -arrestin interaction partners
 - Authors: Valeria Robleto, Ya Zhuo, Adriano Marchese
- Graduate School Poster Session 2022, Milwaukee, WI.
 - Title: Role of sorting nexin adaptor proteins in CXCR4 signaling
 - Authors: Valeria Robleto, Ya Zhuo, Adriano Marchese
- Experimental Biology 2021, Virtual, ASPET
 - Title: Role of sorting nexin adaptor proteins in CXCR4 signaling. (*Selected for poster competition*)
 - Authors: Valeria Robleto, Ya Zhuo, Adriano Marchese
- Graduate School Poster Session 2021, Milwaukee, WI.
 - Title: Role of sorting nexin proteins in CXCR4 signaling
 - Authors: Valeria Robleto, Ya Zhuo, Adriano Marchese
- Experimental Biology 2020, San Diego, CA-ASPET (Cancelled due to covid).
 - Title: Elucidating the signaling pathways of CXCR4-dependent chemotaxis.
 - Authors: Valeria Robleto, Ya Zhuo, Adriano Marchese
- Graduate School Poster Session 2019, Milwaukee, WI.
 - Title: Elucidating Pathways of CXCR4-dependent Chemotaxis
 - Authors: Valeria Robleto, Ya Zhuo, Adriano Marchese
- Arkansas INBRE Conference 2017, Fayetteville, AR.
 - Title: Cloning and Expression of Human Separase. (*Honorable Mention*)
 - Authors: Valeria Robleto, Debananda Pati

PROFESSIONAL MEMBERSHIPS

- *The American Society for Pharmacology and Experimental Therapeutics 2020-Current*
- *The American Heart Society 2021*
- *The American Society of Biochemistry and Molecular Biology 2022*

SERVICE AND LEADERSHIP

- Ambassador for Summer Program of Undergraduate Research, Medical College of Wisconsin, May-July 2023
- Biochemistry Graduate Student Representative, Medical College of Wisconsin, 2021-2022
- Volunteer during Graduate School Interview weekend, 2019, 2020, and 2023.
- Leader for catholic youth group “Venture”, 2018, 2019
- President of Student Government Association, University of the Ozarks, 2017-2018
- Vice-President of Ozarks Biological Society, University of the Ozarks, 2017-2018
- Orientation Leader, University of the Ozarks, fall of 2015, 2016, and 2017
- Vice President of Student Government Association, University of the Ozarks, 2016-2017
- President of Catholic Campus Ministries, University of the Ozarks, 2016-2017
- Vice President of Alchemist (Ozarks Chemical Society), University of the Ozarks, 2016-2017
- Historian of Ozarks Biological Society, University of the Ozarks, 2016-2017