BIOGRAPHICAL SKETCH

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NAME: Maria E. Falzone

eRA COMMONS USER NAME (credential, e.g., agency login): mfalzone15

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
Drew University	B.A.	05/2014	Biochemistry and Molecular Biology
Weill Cornell Graduate School	Ph.D.	10/2019	Biomedical Sciences
Rockefeller University	n/a (Postdoc)	08/2024	Molecular Neurobiology and Biophysics

A. Personal Statement

I am fascinated by membrane-delimited components of signaling pathways and the role of the membrane therein. It has become increasing evident that the plasma membrane plays an active role in signaling processes through changes in its physico-chemical properties mediated by signaling-dependent phospholipase enzymes. These enzymes cleave phospholipids at differing positions, leading to the production of both membrane-embedded and soluble second messengers. Phospholipases are particularly important for receptor-tyrosine kinase (RTK) and G protein-coupled receptor (GPCR) signaling pathways, which regulate a myriad of cellular processes, including growth and metabolism. Accordingly, the dysfunction and dysregulation of these enzymes has been implicated in many human diseases, including cancer. Despite their clear physiological importance, fundamental mechanistic information regarding the regulation of phospholipases by their signaling pathways is lacking. The goal for my independent research program is to understand the molecular mechanisms of regulation of phospholipases by their upstream signaling pathways and how this regulation is disrupted in cancer.

These mechanistic details remain elusive because of challenges associated with incorporating the membrane into biochemical and structural studies. During my postdoctoral training in the lab of Dr. Roderick MacKinnon at Rockefeller University, I developed novel tools to overcome these challenges, which facilitated studying the structure and function of one phospholipase, PLCβ, in the presence of the membrane environment. These studies highlighted the importance of the membrane in phospholipase regulation. Accordingly, work in my independent group will utilize similar tools and develop additional ones to study the phospholipase function and regulation.

My training has prepared me well for this undertaking. I have extensive experience with the expression, purification, and reconstitution of membrane proteins as well as their functional characterization. In addition, I obtained extensive training in structural studies of membrane-associated proteins in both my graduate and postdoctoral projects. Further, the environment at the University of Texas Health at San Antonio (UTHSA) is well-suited to support this project both with infrastructure and collaborations. This project will benefit from access to the institutional cryo-EM facility, the Center for Innovative Drug Discovery (CIDD), and the Optical imaging facility. Additionally, collaborations with colleagues with expertise in cancer cell biology and xenograft models will solidify the investigation into the roles phospholipases in cancer development and progression. Together, my education and the environment at UTHSA are key to complete this work.

1. **Falzone, M.**, Rheinberger, J., Lee, B. C., Peyear, T., Sasset, L., Raczkowski, A., Eng, E., Di Lorenzo, A., Anderson, O. S., Nimigean, C., Accardi, A. Structural basis of Ca^{2+}-dependent activation and lipid transport by a TMEM16 scramblase. *Elife*. 2019,8:e43229, doi: 10.7554/eLife.43229, PMID: 30648972.
2. **Falzone, M.E***., Feng, Z*., Alvarenga, O.E., Pan, Y., Lee, B., Cheng, X., Fortea, E., Scheuring, S., Accardi, A. TMEM16 scramblases thin the membrane to enable lipid scrambling. *Nature Communications.* 2022,13(1):2604. doi: 10.1038/s41467-022-30300-z, PMID: 35562175 *denotes co-first author.
3. **Falzone, M.E**. and MacKinnon, R. $G\beta\gamma$ Activates PIP2 Hydrolysis by Recruiting and Orienting PLCβ on the Membrane Surface. *Proc Natl Acad Sci USA*. 2023,120(20): e2301121120. doi: 10.1073/pnas.2301121120. PMID: 37172014.
4. **Falzone, M.E**. and MacKinnon, R. The mechanism of $G\alpha_q$ regulation of PLCβ3-catalyzed PIP2 hydrolysis. *Proc Natl Acad Sci USA*. 2023,120(48): e2315011120. doi: 10.1073/pnas.2315011120. PMID: 37991948.

Ongoing projects that I would like to highlight include:

Recruitment of First-Time, Tenure-Track Faculty Members
Cancer Prevention and Research Institute of Texas (CPRIT)
Falzone (PI)
07/2024-07/2029
Defining Oncogenic Roles of Lipid Cleavage Enzymes

Recently completed projects that I would like to highlight include:

5F32GM142137-03
Falzone (PI)
05/01/2021-04/30/2024
Activation of phospholipase C beta enzymes by G beta-gamma and corresponding regulation of downstream ion channels

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

Start: 9/2024	Assistant Professor, Department of Biochemistry and Structural Biology, The University of Texas Health Science Center San Antonio (San Antonio, TX)
12/2019-8/2024	Postdoctoral Fellow, Roderick MacKinnon Laboratory, Rockefeller University (New York, NY)
2018-present	Member, Biophyscial Society (BPS)
2018-present	Member, Society of General Physiologists
2013	Summer Undergraduate Research Program Fellow, Dr. Steve Almo Laboratory, Albert Einstein College of Medicine. (New York, NY)
5/2011-12/2013	Undergraduate Researcher, Dr. Arnold Demain Laboratory, Drew University

Honors

2024	Invited Speaker, Gordon Research Conference: Phosphorylation and G-Protein Mediated Signaling Networks. New Hampshire
2024	Invited Speaker, Gordon Research Conference: Ligand Recognition and Molecular Gating. Ventura, California.
2022	National Center for Cryo-EM Access and Training (NCCAT) GUP1: Time awarded.
2022	Invited Speaker, International SMALP meeting. Virtual meeting.
2021	Kirschstein-NRSA postdoctoral fellowship (F32)
2021	Invited Speaker, Biophysical Society Virtual Networking Event. Virtual Meeting.
2018	Invited Speaker, New York Structural Biology Discussion Group Summer Meeting. New York, NY
2018	Margaret & Herman Sokol Fellowship (Weill Cornell Graduate School)

2018	First Place Poster Prize - COMPPA Symposium on Membrane Protein Production & Analysis
2014	B.A. awarded with specialized honors and *summa cum laude* (Drew University)
2014	American Institute of Chemists' Award (Drew University)
2013	George de Stevens Award for research (Drew University)

C. Contributions to Science

Undergraduate training

For my undergraduate thesis, I worked in the laboratory of Dr. Arnold Demain at Drew University and studied the biosynthesis of the natural product antibiotics platensimycin and platencin. Antibiotic resistant pathogens are a significant global health problem, emphasizing the need for new antibiotics. Platensimycin and platencin were discovered by Merck & Co in the early 2000's and are produced by the soil actinomycete *Streptomyces platensis*. They were shown to be active against *Mycobacterium tuberculosis* and gram-positive bacteria including antibiotic resistant strains. These compounds inhibit fatty acid synthesis, specifically the FabF and FabH enzymes which are not present in eukaryotic cells, making them non-toxic to humans. We set out to establish a chemically defined culture medium to facilitate the study of the biosynthetic pathway of these compounds within *S. platensis*. During my time in the lab, I contributed to the development of a semi-defined and fully-defined culture medium for *S. plateneis* that supports production of these compounds. This project laid the groundwork for the follow-up work, which investigated the effects of amino acids and vitamins on antibiotic production. I contributed to showing that addition of aspartic acid to the chemically defined medium increases antibiotic production, consistent with its role in the biosynthetic pathway. This work proved insights into the biosynthesis of platensimycin and platensin by *S. plateneis.*

1. Aluotto S., Tynan H., Maggio C., **Falzone M**., Mukherjee A, Gullo V., Demain AL. Development of a semi-defined medium supporting production of platensimycin and platencin by *Streptomyces platensis*. *Journal of Antibiotics*. 2012,66(2):51-54, doi: 10.1038/ja.2012.97, PMID: 23188381.
2. **Falzone M**., Martens E., Tynan H., Maggio C., Golden S., Nayda V., Crespo E., Inamine G., Gelber M., Lemence R., Chiappini N., Friedman E., Shen B., Gullo V., Demain AL. Development of a chemically defined medium for the production of the antibiotic platensimycin by *Streptomyces platensis*. *Appl Microbiol Biotechnol*. 2013,97(21):9535-9, doi: 10.1007/s00253-013-5201-6, PMID: 24022611.
3. **Falzone, M**., Crespo, E., Jones, K., Khan, G., Korn, V. L., Patel, A., Demain, A. L. Nutritional control of antibiotic production by *Streptomyces platensis* MA7327: Importance of l-aspartic acid. *Journal of Antibiotics*. 2017,70(7):828-831, doi: 10.1038/ja.2017.49, PMID: 28465627. PMCID: PMC5642980.

Graduate Training

I carried out my doctoral dissertation work in the laboratory of Dr. Alessio Accardi studying the TMEM16 phospholipid scramblases. In eukaryotic cells, scramblases flip lipids between the leaflets of the membrane down their concentration gradients. Increases in cytosolic Ca^{2+} activate plasma membrane TMEM16 scramblases, which collapse the lipid asymmetry exposing phosphatidylserine (PS) on the extracellular surface. Externalized PS serves as a signaling initiator for many processes including blood coagulation, and apoptosis, highlighting the importance of scramblases. Using fungal TMEM16 model systems, it was shown that lipids are translocated through a large hydrophilic cavity directly exposed to the membrane. Many TMEM16 scramblases are also non-selective ion channels, in which ions are transported through the same region of the protein as lipids. My contributions are related to understanding the mechanism of scrambling, Ca^{2+}-dependent gating, and modulation by membrane properties.

Using cryo-EM, I determined structures of a fungal TMEM16 in a membrane environment in the presence and absence of Ca^{2+}. The structures showed that Ca^{2+}-dependent gating occurs via movements in two helices that are part of the permeation pathway. Through a collaboration with the lab of Dr. Harel Weinstein at Weill Cornell Medicine, we showed that a hydrophobic lock between two pathway helices also plays a role in gating, including regulating the equilibrium between a scramblase and channel-only conformation. Through a collaboration with the laboratory of Dr. Elisabeth Carpenter at Oxford, I characterized the function of hTMEM16K *in vitro,* which is

localized in the endoplasmic reticulum, making its characterization in cells challenging. I showed that hTMEM16K is a scramblase and non-selective channel with properties very similar to the fungal model systems. Accompanying structures revealed that the Ca^{2+}-dependent gating mechanism is conserved in some mammalian scramblases.

In addition, my structures of the fungal scramblase in a membrane environment revealed that TMEM16 scramblases disrupt the organization of the surrounding membrane, inducing a pronounced bending. Additional, higher resolution structures revealed specific protein-lipid interactions providing molecular details to the observed membrane bending. Accompanying *in vitro* functional experiments showed that scrambling is inhibited by thicker membranes and by ceramide lipids which rigidify the membrane, consistent with the observed altered membrane organization. The mechanism of this modulation was further investigated to reveal that it is not dependent on conformational changes of the protein but rather changes in lipid permeation. Additional functional experiments suggest that lipids do not need to enter the scrambling cavity to be transported, challenging the paradigm of the credit card mechanism in the field. We proposed that scrambling requires local membrane thinning imposed by the scramblase and in environments that reduce thinning, like thicker or more rigid bilayers, scrambling is reduced.

1. **Falzone, M.**, Rheinberger, J., Lee, B. C., Peyear, T., Sasset, L., Raczkowski, A., Eng, E., Di Lorenzo, A., Anderson, O. S., Nimigean, C., Accardi, A. Structural basis of Ca2+-dependent activation and lipid transport by a TMEM16 scramblase. *Elife*. 2019,8:e43229, doi: 10.7554/eLife.43229, PMID: 30648972.
2. Khelashvili, G*., **Falzone, M***., Cheng, X., Lee, B. C., Accardi, A., Weinstein, H. Dynamic modulation of the lipid translocation groove generates a conductive ion channel in Ca2+-bound nhTMEM16. *Nature Communications*. 2019,10(1):4972. doi: 10.1038/s41467-019-12865-4, PMID: 31672969.
3. Bushell, S. R*., Pike, A. C. W*., **Falzone, M*.,** Rorsman, N. J. G., Ta, C. M., Corey, R. A., Newport, T. D., Shintre, C. A., Tessitore, A., Chu, A., Wang, Q., Shrestha, L., Mukhopadhyay, S. M. M., Love, J., Burgess-Brown, N. A., Sitsapesan, R., Sttansfeld, P. J., Huiskonen, J. T., Tammaro, P., Accardi, A., Carpenter, E. P. The structural basis of lipid scrambling and inactivation in the endoplasmic reticulum scramblase TMEM16K. *Nature Communications*. 2019,10(1):3956. doi: 10.1038/s41467-019-11753-1, PMID: 31477691.
4. **Falzone, M.E***., Feng, Z*., Alvarenga, O.E., Pan, Y., Lee, B., Cheng, X., Fortea, E., Scheuring, S., Accardi, A. TMEM16 scramblases thin the membrane to enable lipid scrambling. *Nature Communications.* 2022,13(1):2604. doi: 10.1038/s41467-022-30300-z, PMID: 35562175 *denotes co-first author.

Postdoctoral training

I completed postdoctoral training in the lab of Dr. Roderick MacKinnon at Rockefeller University studying the regulation of PLCβ enzymes by G proteins. PLCβ cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) producing inositol triphosphate (IP3) and diacylglycerol (DAG), which mediate a myriad of downstream responses. It is regulated by the G proteins Gβγ and $G\alpha_q$, but the mechanisms were not well understood. Progress in understanding the regulation of phospholipases has been hampered by challenges associated with studying the structure and function of these proteins. PLCβ, like most phospholipases, are aqueous-soluble but must partition onto the membrane surface to access their lipid substrates, allowing them to be regulated at the partitioning step, the catalytic step, or both. Despite the crucial role of the membrane in phospholipase regulation, it is often omitted from structural and functional characterization due to complications associated with including it. To overcome these challenges, I developed novel tools to study PLCβ structure and function, including an electrophysiology based functional assay using a PIP2-dependent ion channel as a read out, a membrane partitioning assay, and a workflow to determine structures of PLCβ-G protein on the membrane surface. Separating the partitioning and catalytic steps was essential to unraveling the mechanism of regulation of PLCβ by G proteins. Using these tools, I showed that Gβγ and $G\alpha_q$ activate PLCβ by differing mechanisms, Gβγ by recruitment of PLCβ to the membrane and $G\alpha_q$ by enhancing the catalytic rate constant. Structural studies yielded the first PLCβ-Gβγ structure and revealed that two Gβγ bind to PLCβ and anchor its catalytic core to the membrane. These studies laid the groundwork for the proposed project and provided me with invaluable

experience with the necessary methods. These tools will be easily adaptable to study other phospholipases by utilizing different ion channels that are gated by the substrate or products.

1. **Falzone, M.E**. and MacKinnon, R. Gβγ Activates PIP2 Hydrolysis by Recruiting and Orienting PLCβ on the Membrane Surface. *Proc Natl Acad Sci USA*. 2023,120(20): e2301121120. doi: 10.1073/pnas.2301121120. PMID: 37172014.
2. **Falzone, M.E**. and MacKinnon, R. The mechanism of $G\alpha_q$ regulation of PLCβ3-catalyzed PIP2 hydrolysis. *Proc Natl Acad Sci USA*. 2023,120(48): e2315011120. doi: 10.1073/pnas.2315011120. PMID: 37991948.

Complete list of Published work:
https://pubmed.ncbi.nlm.nih.gov/?term=Falzone%20Maria&sort=date&pos=9