BIOGRAPHICAL SKETCH

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NAME: Fu, Ziao

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

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
Jilin University, Changchun, China	B.S.	05/2012	Chemistry
Stony Brook University, Stony Brook, NY	M.S.	05/2014	Chemistry
Columbia University, New York, NY	Ph.D.	05/2019	Biological Science
Rockefeller University, New York, NY	Postdoctoral	09/2024	Molecular Neurobiology and Biophysics

A. Personal Statement

I am an Assistant Professor of Cell Biology & Physiology at Washington University in St. Louis, and my research centers on understanding how membrane proteins sense and respond to mechanical forces. My long-term interest is in elucidating the molecular mechanisms underlying mechanosensitivity, specifically focusing on the mechanosensitive ion channel Piezo1. Piezo1 is a critical membrane protein that translates mechanical stimuli into cellular signals, governing key physiological processes such as vascular development and red blood cell volume regulation. However, the structural dynamics of Piezo1 in its native membrane environment remain incompletely understood. My goal is to address these gaps using state-of-the-art cryo-electron microscopy (cryo-EM) techniques. I have a robust background in structural biology and cryo-EM, with expertise in both high-resolution structural analysis and innovative sample preparation. During my Ph.D. training with Dr. Joachim Frank at Columbia University, I developed proficiency in cryo-EM methods, which I expanded further as a postdoctoral researcher in Dr. Rod MacKinnon's lab. There, I established a unique approach to studying membrane proteins directly in their native lipid bilayers, bypassing the need for detergents that often disrupt crucial interactions. This strategy enabled me to investigate Piezo1's conformational changes in response to varying membrane curvatures, providing foundational insights into its mechanosensory function.

My recent work demonstrated that Piezo1 transitions between distinct curvature states, driven by the surrounding membrane environment. Using cryo-EM and cryo-electron tomography, I captured different conformations of Piezo1 in liposomes of varying sizes, revealing the molecular basis of its force transduction. This research provides a structural framework for understanding how Piezo1 senses mechanical forces and links these changes to its physiological roles. The proposed project will build on this knowledge, aiming to refine the 6.1 Å Piezo1 structure to uncover critical structural details, such as pore expansion, that are essential for its gating mechanism. My experience in cryo-EM and mechanosensory proteins uniquely positions me to lead this project and generate meaningful insights into Piezo1's mechanotransduction. At Washington University, I have access to world-class facilities and a collaborative scientific environment that will support the proposed research. With my expertise in cryo-EM and membrane protein biology, I am confident that this project will yield high-impact results that will deepen our understanding of Piezo1's mechanosensitivity. My commitment to advancing mechanobiology, combined with my passion for mentoring the next generation of scientists, will ensure that the outcomes of this research are both scientifically rigorous and broadly impactful.

Citations:

1. Haselwandter, C. A.; Guo, Y. R.; **Fu, Z.**; MacKinnon, R. Quantitative Prediction and Measurement of Piezo's Membrane Footprint. Proc. Natl. Acad. Sci. 2022, 119 (40), e2208027119. PMCID: PMC9546538
2. Haselwandter, C. A.; Guo, Y. R.; **Fu, Z.**; MacKinnon, R. Elastic Properties and Shape of the Piezo Dome Underlying Its Mechanosensory Function. Proc. Natl. Acad. Sci. 2022, 119 (40), e2208034119. PMCID: PMC9546593
3. **Fu, Z.** & MacKinnon, R. Structure of the Flotillin Complex in a Native Membrane Environment. Proc. Natl. Acad. Sci. 2024, 121 (29), e2409334121. PMCID: PMC11260169

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

10/2024 - Assistant Professor, Department of Cell Biology & Physiology, Washington University, St. Louis, MO

Honors

2019 Titus M. Coan Prize for Excellence in Research (Basic Cell and Molecular Biology)
2019 Best Poster Award The 7th International Ion Channel Conference
2018 COMPPÅ Symposium Fisher Award

Professional Societies and Organizations

2014 - Biophysical Society

C. Contributions to Science

1. My early graduate research focused on the aggregation of amyloid-β (Aβ) peptides, a key process in Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA). Using advanced biophysical techniques, I discovered that Aβ initially forms unstructured low-molecular-weight oligomers that stack into more toxic forms, leading to protofibril formation. Importantly, I found that curcumin and resveratrol, natural compounds from curry and red wine, can bind to these early oligomers, preventing their toxic progression. Additionally, I demonstrated that familial mutations in Aβ peptides, linked to early-onset AD and CAA, lead to unique structural intermediates and that preexisting amyloid can influence the aggregation of Aβ monomers into disease-specific structures. These findings provide crucial insights into the molecular mechanisms of these neurodegenerative diseases and suggest new therapeutic targets. My role encompassed designing and conducting experiments, as well as interpreting the data to connect these structural changes to disease pathology.

   a. **Fu, Z.**; Aucoin, D.; Ahmed, M.; Ziliox, M.; Van Nostrand, W. E.; Smith, S. O. Capping of Aβ42 Oligomers by Small Molecule Inhibitors. Biochemistry 2014, 53 (50), 7893–7903. PMCID: PMC4278677
   b. **Fu, Z.**; Aucoin, D.; Davis, J.; Van Nostrand, W. E.; Smith, S. O. Mechanism of Nucleated Conformational Conversion of Aβ42. Biochemistry 2015, 54 (27), 4197–4207. PMID: 26069943
   c. Xu, F.*; **Fu, Z.***; Dass, S.; Kotarba, A. E.; Davis, J.; Smith, S. O.; Van Nostrand, W. E. Cerebral Vascular Amyloid Seeds Drive Amyloid β-Protein Fibril Assembly with a Distinct Anti-Parallel Structure. Nat. Commun. 2016, 7 (1), 13527. PMCID: PMC5121328
   d. **Fu, Z.***; Crooks, E. J.*; Irizarry, B. A.; Zhu, X.; Van Nostrand, W. E.; Chowdury, S.; Smith, S. O. An Electrostatic Cluster Guides Aβ40 Fibril Formation in Cerebral Amyloid Angiopathy. J. Struct. Biol. 2024 Apr 13;216(2):108092. PMCID: PMC11162928

2. The study of short-lived intermediates in biological processes, such as bacterial translation, has historically been limited by the inability to capture these fleeting states at high resolution. In 2014, during my Ph.D. research in Joachim Frank's lab at Columbia University, I focused on optimizing time-resolved cryo-electron microscopy (cryo-EM) to overcome this challenge. My work centered on capturing and analyzing intermediate states during key stages of bacterial translation, including ribosome recycling, translation initiation, and termination. The central findings of my research were the discovery and high-resolution characterization of previously unknown intermediate states in these processes. These discoveries provided critical insights into the molecular mechanisms of translation and have significant implications for the development of new

antibiotics. My role involved the technical optimization of the time-resolved cryo-EM method, designing experiments, and interpreting the structural data, which collectively advanced the application of this powerful technique to broader biological questions.

a. **Fu, Z.***; Kaledhonkar, S.*; Borg, A.*; Sun, M.; Chen, B.; Grassucci, R. A.; Ehrenberg, M.; Frank, J. Key Intermediates in Ribosome Recycling Visualized by Time-Resolved Cryoelectron Microscopy. Structure 2016, 24 (12), 2092–2101. PMCID: PMC5143168
b. Feng, X.*; **Fu, Z.***; Kaledhonkar, S.; Jia, Y.; Shah, B.; Jin, A.; Liu, Z.; Sun, M.; Chen, B.; Grassucci, R. A.; Ren, Y.; Jiang, H.; Frank, J.; Lin, Q. A Fast and Effective Microfluidic Spraying-Plunging Method for High-Resolution Single-Particle Cryo-EM. Structure 2017, 25 (4), 663-670.e3. https://doi.org/10.1016/j.str.2017.02.005. PMCID: PMC5382802
c. **Fu, Z.***; Indrisiunaite, G.*; Kaledhonkar, S.*; Shah, B.; Sun, M.; Chen, B.; Grassucci, R. A.; Ehrenberg, M.; Frank, J. The Structural Basis for Release-Factor Activation during Translation Termination Revealed by Time-Resolved Cryogenic Electron Microscopy. Nat. Commun. 2019, 10 (1), 2579. PMCID: PMC6561943
d. Kaledhonkar, S.*; **Fu, Z.***; Caban, K.*; Li, W.; Chen, B.; Sun, M.; Gonzalez, R. L.; Frank, J. Late Steps in Bacterial Translation Initiation Visualized Using Time-Resolved Cryo-EM. Nature 2019, 570 (7761), 400–404. PMCID: PMC7060745

3. The activation mechanisms of Class-C G protein-coupled receptors (GPCRs) have long been a challenging area in biomedical research due to their complex structures and roles in various physiological processes. During my Ph.D. training, I collaborated with Dr. Qing R. Fan, a leading expert in Class-C GPCRs at Columbia University, to address these challenges. My research focused on elucidating the structures of key Class-C GPCRs in different functional states. I successfully determined the structure of the human calcium-sensing receptor (CaSR) in its active state, uncovering symmetric activation and modulation mechanisms with significant implications for calcium-related therapies. Additionally, I characterized the structure of the human GABAB receptor in its inactive state, providing crucial insights for the development of drugs aimed at treating neurological disorders such as epilepsy and depression. These findings advance our understanding of Class-C GPCR activation and regulation, opening new avenues for therapeutic interventions in both calcium-related and neurological diseases. My specific role in this work included designing experiments, solving the receptor structures, and interpreting the data to reveal the mechanisms underlying their activation and regulation.

a. Park, J.*; **Fu, Z.***; Frangaj, A.*; Liu, J.*; Mosyak, L. *; Shen, T. *; Slavkovich, V. N.; Ray, K. M.; Taura, J.; Cao, B.; Geng, Y.; Zuo, H.; Kou, Y.; Grassucci, R.; Chen, S.; Liu, Z.; Lin, X.; Williams, J. P.; Rice, W. J.; Eng, E. T.; Huang, R. K.; Soni, R. K.; Kloss, B.; Yu, Z.; Javitch, J. A.; Hendrickson, W. A.; Slesinger, P. A.; Quick, M.; Graziano, J.; Yu, H.; Fiehn, O.; Clarke, O. B.; Frank, J.; Fan, Q. R. Structure of Human GABAB Receptor in an Inactive State. Nature 2020, 584 (7820), 304–309. PMCID: PMC7725281
b. Park, J.*; Zuo, H.*; Frangaj, A.*; **Fu, Z.***; Yen, L. Y.*; Zhang, Z.*; Mosyak, L.; Slavkovich, V. N.; Liu, J.; Ray, K. M.; Cao, B.; Vallese, F.; Geng, Y.; Chen, S.; Grassucci, R.; Dandey, V. P.; Tan, Y. Z.; Eng, E.; Lee, Y.; Kloss, B.; Liu, Z.; Hendrickson, W. A.; Potter, C. S.; Carragher, B.; Graziano, J.; Conigrave, A. D.; Frank, J.; Clarke, O. B.; Fan, Q. R. Symmetric Activation and Modulation of the Human Calcium-Sensing Receptor. Proc. Natl. Acad. Sci. 2021, 118 (51), e2115849118. PMCID: PMC8713963

4. The mechanosensitive ion channel Piezo1 plays a vital role in sensing membrane tension, yet understanding its structural mechanism in native-like environments has been a challenge. In 2019, I joined Rod MacKinnon's lab to address this problem, leveraging my expertise in cryo-electron microscopy (cryo-EM) to study membrane proteins in more native settings. Previous studies showed that Piezo1, when stabilized in detergents, exhibited a highly curved structure, but how this related to its function within a natural lipid bilayer was unclear. To explore this, I reconstituted Piezo1 into liposomes, which more accurately simulate the native lipid bilayer environment. My central finding was that Piezo1 induces tear-drop shapes in spherical liposomes, with the surrounding curved membrane exerting forces on the channel. By analyzing vesicles of varying sizes, I demonstrated that Piezo1 adopts different shapes based on the membrane forces, allowing for the calculation of its stiffness. Using cryogenic electron tomography (cryo-ET), I obtained precise 3D shapes of Piezo1 vesicles and discovered that Piezo1 is less curved in planar lipid bilayers than in detergent micelles, revealing that its rigidity is comparable to that of a free lipid bilayer. These insights provide a crucial framework for understanding how proteins deform bilayer membranes and enhance our understanding of Piezo1's

mechanosensory function. My specific role involved reconstituting Piezo1 in liposomes, conducting cryo-EM and cryo-ET analyses, and interpreting the data to elucidate the structural dynamics of Piezo1 in its native-like environment.

a. Haselwandter, C. A.; Guo, Y. R.; **Fu, Z.**; MacKinnon, R. Quantitative Prediction and Measurement of Piezo's Membrane Footprint. Proc. Natl. Acad. Sci. 2022, 119 (40), e2208027119. PMCID: PMC9546538
b. Haselwandter, C. A.; Guo, Y. R.; **Fu, Z.**; MacKinnon, R. Elastic Properties and Shape of the Piezo Dome Underlying Its Mechanosensory Function. Proc. Natl. Acad. Sci. 2022, 119 (40), e2208034119. PMCID: PMC9546593

5. The formation of membrane microdomains, essential for various cellular processes such as signaling and trafficking, has been a complex area of study, particularly in understanding the role of the Flotillin complex. Historically, structural characterization of such membrane proteins has been hindered by the need for detergents, which can disrupt native protein-lipid interactions. In this study, I aimed to overcome these challenges by elucidating the structure of the Flotillin complex within its native membrane environment. Utilizing advanced cryo-electron microscopy combined with a novel membrane protein stabilization method, I successfully resolved the structure of the Flotillin complex without the use of detergents or overexpression, preserving its natural conformation and interactions with the surrounding lipid bilayer. The central finding of this research was the detailed architecture of the Flotillin complex, which provided new insights into its role in inducing membrane curvature and organizing microdomains. This understanding is crucial for elucidating how Flotillin proteins influence processes such as clathrin-independent endocytosis. The ability to study the Flotillin complex in its native state represents a significant advancement in membrane biology and paves the way for future research into the functional roles of SPFH family proteins and their implications in various cellular processes and disease mechanisms. My specific role in this work included designing the experimental approach, conducting cryo-EM analysis, and interpreting the data to uncover the structural dynamics of the Flotillin complex in its native environment.

a. **Fu, Z.**; MacKinnon, R. Structure of the Flotillin Complex in a Native Membrane Environment. Proc. Natl. Acad. Sci. 2024, 121 (29), e2409334121. PMCID: PMC11260169

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