

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, specializing in cryo-electron microscopy (cryo-EM) to study protein assemblies and membrane complexes in their native environments. This proposal focuses on resolving the high-resolution structure of flat amyloid fibrils, a structurally diverse and disease-relevant subset of fibrils, to uncover their spatial organization, molecular interactions, and roles in amyloid-driven diseases. My research aims to elucidate how the structural and spatial properties of amyloid fibrils contribute to their pathogenicity and to generate insights that inform therapeutic strategies for conditions such as Alzheimer's disease and cerebral amyloid angiopathy (CAA). I have extensive research experience in the beta-amyloid field, including work on CAA-related amyloid fibril structures. My previous studies employed cryo-EM to resolve the structures of fibrils derived from patient tissues, shedding light on their polymorphic nature and pathological relevance. These efforts provided critical insights into how fibril structure relates to disease progression and severity. During my Ph.D. with Dr. Joachim Frank at Columbia University, I contributed to advancing cryo-EM methodologies, and during my postdoctoral training with Dr. Rod MacKinnon, I developed approaches to study complex protein assemblies directly within native environments. This combination of experience has equipped me to tackle challenging structural biology questions, including the characterization of non-helical flat amyloid fibrils. This proposal employs advanced cryo-EM workflows and the Chameleon system to overcome orientation bias, a significant challenge in studying flat fibrils using traditional blotting methods. By leveraging these cutting-edge tools, we aim to achieve high-resolution structural reconstructions of flat amyloid fibrils and uncover their mechanistic roles in disease pathology. This study builds on my prior work and expands our understanding of the structural underpinnings of amyloid-related diseases. With access to state-of-the-art resources at Washington University and NCCAT's advanced cryo-EM facility, and a lab uniquely equipped to address these questions, I am well-positioned to lead this project. The insights gained will deepen our understanding of amyloid fibrils in the context of Alzheimer's disease and CAA, advancing the development of innovative therapeutic approaches and contributing to the broader field of amyloid research.

Citations:

1. **Fu, Z.***; Crooks, E. J.*; Irizarry, B. A.; Zhu, X.; Van Nostrand, W. E.; Chowdury, S.; Smith, S. O. An Electrostatic Cluster Guides A β 40 Fibril Formation in Cerebral Amyloid Angiopathy. *J. Struct. Biol.* 2024 Apr 13;216(2):108092. PMID: PMC11162928

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 COMPPA 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 (A β) peptides, a key process in Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA). Using advanced biophysical techniques, I discovered that A β 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 A β peptides, linked to early-onset AD and CAA, lead to unique structural intermediates and that preexisting amyloid can influence the aggregation of A β 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 A β 42 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 A β 42. *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 A β 40 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. PMID: 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. PMID: 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. PMID: 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. PMID: 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. PMID: 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. PMID: 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. PMID: PMC11260169

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/ziao.fu.1/bibliography/public/>