BIOGRAPHICAL SKETCH

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NAME: Fu, Ziao

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

POSITION TITLE: Postdoctoral Associate

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	B.S.	05/2012	Chemistry
Stony Brook University	M.S.	05/2014	Chemistry
Columbia University	Ph.D.	05/2019	Biological Science
Rockefeller University	Postdoctoral Associate	-	Biophysics

A. Personal Statement

Our lab has developed methods for determining the structure of membrane proteins directly from their cellular environment, preserving their native state. Utilizing these techniques, I explored the potential to identify endogenous membrane proteins without over-expression or enrichment. During my analysis of plasma membrane vesicles from HEK293 cells using cryo-EM, I discovered large, truncated cone-shaped objects attached to the membranes, identified as Flotillin cages. These structures are composed of Flotillin1 and Flotillin2, mammalian SPFH proteins. Building on the findings of the first SPFH family protein structure in 2022, my investigation into Flotillin cages revealed similar membrane-bound ring-like architectures observed in other SPFH family members and related proteins. My study not only advances understanding of Flotillin's structural role but also its potential functions in organizing membrane proteins and influencing mechanosensory channels, pertinent for various cellular processes. This work underscores the broader implications of our novel preparation methods in uncovering new insights into membrane protein biology.

B. Positions, Scientific Appointments, and Honors

Positions

2019- Postdoctoral Associate, Laboratory of Molecular Neurobiology and Biophysics, Rockefeller University, New York, NY

Awards and Honors

2018 COMPPÅ Symposium Fisher Award
2019 The 7th International Ion Channel Conference Best Poster Award
2019 Titus M. Coan Prize for Excellence in Research (Basic Cell and Molecular Biology)
2020 The Chinese Government Award for Outstanding Students Abroad

C. Contributions to Science

1. Mechanism of mechanosensitive ion channels Piezo1. The detergent-stabilized Piezo1 structure was found to be highly curved. Because it is a mechanosensitive channel and it senses lipid tension, and to better understand its mechanism, I reconstituted Piezo1 into liposomes. I reasoned that when a Piezo resides in a lipid bilayer liposome, it changes spherical liposomes into tear-drop liposomes; the curved lipid membrane surrounding the channel also exerts a force on it. Vesicles of different sized exert different amounts of force, causing Piezo1 to adopt a distinct shape in each. Considering Piezo as a spring, by knowing the displacement and the force applied, we can calculate the stiffness of Piezo1. I gathered cryogenic electron tomography data to determine the accurate 3D shape of Piezo1 vesicles. I found that Piezo is less curved in planar lipid bilayer membranes than in detergent micelles, and it has a rigidity similar to a free lipid bilayer membrane. These findings give a broad framework for characterizing how proteins deform bilayer membranes.

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. https://doi.org/10.1073/pnas.2208027119.

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. https://doi.org/10.1073/pnas.2208034119.

2. Time-resolved cryo-EM development and application. In 2014, I joined Joachim Frank's group at Columbia University as a PhD student and concentrated on optimizing time-resolved cryo-EM to investigate short-lived intermediates during certain steps of bacterial translation. During my studies of ribosome recycling, translation initiation, and termination using time-resolved cryo-EM, I discovered new intermediate states and was able to characterize their structures to high resolution. These findings provide insight into the development of antibiotics and set the stage for future application of time-resolved cryo-EM to other biological processes.

a. 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. https://doi.org/10.1038/s41586-019-1249-5.

b. **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. https://doi.org/10.1038/s41467-019-10608-z.

c. 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.

d. **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. https://doi.org/10.1016/j.str.2016.09.014.

3. Mechanism of assembly and inhibition of Aβ peptide. Using a combination of biophysical approaches to probe the structural transitions in fibril formation—namely Nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, and atomic force microscopy—I discovered that the Aβ peptide clusters into highly unstructured low-molecular weight (MW) oligomers that can stack to produce high-MW oligomers that laterally associate to form protofibrils. Importantly, I showed that the high-MW oligomers are more toxic than the low-MW oligomers. Curcumin and resveratrol (natural product extracts from curry and red wine, respectively) bind to the N-terminus of the low-MW forms and limit their transition to the toxic high-MW forms. These findings not only shed light on the Aβ aggregation pathway but also suggest a way to prevent the formation of toxic Aβ species. After studying the aggregation pathway from monomers to protofibrils, I investigated the transition of Aβ peptides containing familial mutations. Patients bearing these mutations usually develop AD and CAA at an early age. My research focused on familial mutations in CAA, which is characterized by cerebrovascular Aβ accumulation and results in vascular cognitive impairment and dementia. I demonstrated that familial mutant peptides form transitory intermediates with an antiparallel β-structure, whereas wild-type Aβ normally adopts a parallel β-sheet structure. Furthermore, I discovered that isolated microvascular amyloid from CAA mutant transgenic mice models induces the assembly of human wild-type Aβ

into unique antiparallel-β-sheet fibrils. These findings suggest that preexisting amyloid can drive the Aβ monomer to aggregate into specific structures, which may contribute to distinct pathologies and provide insight into the development of AD and CAA.

a. **Fu, Z.**; Van Nostrand, W. E.; Smith, S. O. Anti-Parallel β-Hairpin Structure in Soluble Aβ Oligomers of Aβ40-Dutch and Aβ40-Iowa. **Int. J. Mol. Sci.** 2021, 22 (3). https://doi.org/10.3390/ijms22031225.

b. 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. https://doi.org/10.1038/ncomms13527.

c. **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. https://doi.org/10.1021/acs.biochem.5b00467.

d. **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. https://doi.org/10.1021/bi500910b.

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