BIOGRAPHICAL SKETCH

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NAME: Zhao, Chen

eRA COMMONS USER NAME (credential, e.g., agency login): CHEN.ZHAO1

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
University of Science and Technology of China	B.S.	07/2012	Biological Sciences
Yale University	Ph.D.	12/2017	Molecular Biophysics and Biochemistry
The Rockefeller University	Postdoctoral	07/2023	Biochemistry, Structural Biology and Physiology

A. Personal Statement

My long-term career goal is to decipher how membrane receptors and channels modulate signal transduction pathways. My research team approaches this goal by determining the structure of the cell membrane: What are the structures of individual membrane proteins and their complexes? How do different membrane proteins organize into signaling domains by weak molecular interactions? To determine the compositions and structures of the signaling complexes, we will combine structural biology approaches with mass-spectrometry analyses, and place particular emphasis on direct measurements from the native membrane environment. We will also utilize biochemistry, electrophysiology, and cell biology tools to elucidate how the unique composition and structure of the signaling complex lead to its specific physiological function. Finally, because many membrane proteins are drug targets, we will leverage the mechanistic knowledge to facilitate the development of innovative therapeutic tools targeting these membrane proteins.

My scientific training has prepared me to lead research projects that align with my long-term goal. During my postdoc training with Dr. Roderick MacKinnon, I characterized the molecular mechanisms of four different membrane proteins (K_{ATP}, TRPM3, Slo1, GIRK) by cryo-EM and electrophysiology, with three of these studies published. Most importantly, I developed the idea of isolating membrane proteins while keeping them inside the cell membrane via small vesicles. Guided by this idea, we established novel methods that enable high-resolution membrane protein structure determination in the cell membrane environment. In my recently established research group, we will advance this new method and combine it with traditional membrane protein structural biology approaches to determine the molecular mechanisms underlying a specific ion channel, TRPM3. In the long term, we will further extend this method and develop new methods to study the structures and organizations of other membrane receptors or channels involved in signal transduction.

In addition to research, I have also gained substantial exposure to mentoring. During my graduate training, I have mentored two Ph.D. students and one high school student. During my postdoc training, I have mentored two Ph.D. students and two junior postdocs who are new to the field. My role as a mentor includes hands-on technical training, experimental design, project design, and manuscript/proposal editing. During my graduate school, I also volunteered for a “Science Saturday” event where I demonstrated structural biology principles to high school students, especially those from underrepresented backgrounds.

Currently, at the University of Florida, I am mentoring 2 Ph.D. students, 1 master’s student, 1 post-baccalaureate technician, and 2 undergraduate students. In the past year, I have mentored a post-baccalaureate technician seeking additional research experience before applying to graduate school. He has now successfully

received multiple offers and is now pursuing a Ph.D. in his dream program. Additionally, I mentored a high school student through a summer program initiated by the Center for Precollegiate Education and Training at the University of Florida. Over two months, she developed significant skills in both laboratory work and writing. By the time she completed the program, she was able to carry out protein purification independently. She will be applying to the undergraduate program at the University of Florida. I also co-mentored a postdoc with Dr. Michelle Gumz to upgrade the electrophysiology rig in the Gumz lab that is shared with me.

Besides mentoring in the lab, I also serve on the supervisory committee of 5 Ph.D. students and 1 master's student. As a new faculty member in the Department of Biochemistry & Molecular Biology, I hope to bring more motivated students from traditionally underrepresented backgrounds to our department. Therefore, I will be visiting Primarily Undergraduate Institutes and community colleges every year to encourage undergraduate students to join our program and my lab. In 2024, I visited the University of North Florida (a PUI) as part of this outreach effort.

Ongoing and recently completed projects that I would like to highlight include:
N/A

Citations:

1. **Zhao C** and MacKinnon R. Molecular structure of an open human KATP channel. ***PNAS***. 118 (48) e2112267118. **2021**. PMCID: PMC8640745

2. **Zhao C** and MacKinnon R. Structural and functional analyses of a GPCR-inhibited ion channel TRPM3. ***Neuron***. 111: 81–91. **2023**. PMID: 36283409

3. Tao X*, **Zhao C*** and MacKinnon R. Membrane protein isolation and structure determination in cell-derived membrane vesicles. ***PNAS***. 120 (18) e2302325120. **2023**. PMCID: PMC10160969

*equal contribution

4. **Zhao C**. Limitations in membrane protein structure determination by lipid nanodiscs. ***Trends Biochem Sci***. 26:S0968-0004(24)00076-8. **2024**. PMID: 38538407

B. Positions, Scientific Appointments, and Honors

Positions

08/2023 – present	Assistant Professor, Dept of Biochemistry & Molecular Biology, University of Florida College of Medicine
09/2022 – 07/2023	Associate 04, HHMI, The Rockefeller University
09/2021 – 09/2022	Postdoctoral Associate, HHMI, The Rockefeller University
08/2018 – 08/2021	Jane Coffin Childs Fellow, HHMI, The Rockefeller University
09/2017 – 07/2018	Postdoctoral Associate, The Rockefeller University
09/2012 – 08/2017	Graduate Fellow, HHMI, Yale University
09/2006 – 07/2008	Undergraduate Research Assistant, University of Science and Technology of China

Scientific Appointments

2017 – present	*Ad hoc* reviewer for journals including *Structure*, *eLife*, *Journal of Biological Chemistry*, and more.

Honors

04/2024	Nominee from the University of Florida for the Pew Scholars
09/2023	Nominee from the University of Florida for the Searle Scholars Program
08/2018 – 08/2021	Jane Coffin Childs Fellowship
09/2013	Nominee from Yale University for the HHMI International Student Research Fellowships
09/2012 – 09/2015	Gruber Science Fellowship, Yale University

06/2012	Commencement Speech, University of Science and Technology of China
06/2012	Muoruo Guo Fellowship, University of Science and Technology of China
2008 – 2012	Outstanding Student Award, University of Science and Technology of China

C. Contributions to Science

1. Structural and functional characterization of ion channel TRPM3 and its regulation by Gβγ proteins.

TRPM3 is a multi-functional ion channel implicated in various neurological processes. In the dorsal root ganglion, TRPM3 resides in the nociceptor neurons, and its activity leads to pain sensation. TRPM3 is immediately responsible for opioid-mediated analgesics through direct inhibition by the Gβγ proteins released from activated μ-opioid receptors. My research has advanced our understanding of TRPM3 in multiple aspects. First, using cryo-EM, I determined the first and only structure of the TRPM3 channel by itself and in complex with modulators such as PIP_2 lipid and Gβγ protein. These structures opened the opportunities for interpreting TRPM3 physiology and pathology at atomic scales and provided an avenue for structure-based drug development. In addition, I measured the affinity between Gβγ proteins and TRPM3 in cells, which revealed a 20,000-fold higher affinity than the one between Gβγ and another known ion channel target called the GIRK channel. The difference in affinity among different Gβγ targets suggests that diverse mechanisms underlie signaling specificity and potency in distinct pathways. One long-term objective in the lab is to understand how the same effector, such as the Gβγ protein, is able to achieve signaling specificity and manipulate multiple downstream targets (e.g. TRPM3 and GIRK) under distinct physiological conditions.

Additionally, the affinity between Gβγ proteins and TRPM3 in cells is ~200-fold higher than the affinity between Gβγ proteins and TRPM3 purified in detergent. This discrepancy suggests that detergents, despite being the most popular reagent for membrane protein structure determination, are limited in preserving the native states of membrane proteins under physiological conditions. This observation prompted me to develop novel methods for characterizing membrane protein structures in close-to-native environments.

a. **Zhao C** and MacKinnon R. Structural and functional analyses of a GPCR-inhibited ion channel TRPM3. ***Neuron***. 111: 81–91. **2023**. PMID: 36283409

2. Structure determination of membrane proteins in native cell membrane environment.

Traditionally, membrane protein structure determination relies on solubilization and stabilization of membrane proteins by disruptive reagents such as detergents or polymers. These reagents permitted the visualization of many membrane protein structures at atomic resolution. However, the physiochemical properties of detergents and polymers differ substantially from the lipid bilayer membrane. In addition, detergents and polymers cannot preserve weakly bound lipid and protein molecules due to their disruptive nature, eliminating these endogenous factors from structural analyses. These limitations lead to poor correlations between structural and functional observations for many membrane proteins. For example, in my own work on ion channel TRPM3, the affinities between Gβγ proteins and TRPM3 are ~200-fold higher in cells than in detergent.

In this work, in collaboration with a senior scientist in my postdoc lab, we developed affinity-based methods to isolate cell membrane-derived vesicles containing a specific ion channel Slo1. We determined high-resolution structures of the Slo1 channel in both the total membrane and the plasma membrane vesicles using cryo-EM. Particularly, in the plasma membrane vesicles, we discovered a new lipid-binding pocket and a novel ion-binding site in Slo1. This method allows structural analysis of membrane proteins at atomic resolution in near-native cellular environment. We anticipate that this method will enable discoveries of new biology for many other membrane proteins.

a. Tao X*, **Zhao C*** and MacKinnon R. Membrane protein isolation and structure determination in cell-derived membrane vesicles. ***PNAS***. 120 (18) e2302325120. **2023**. PMCID: PMC10160969
*equal contribution

b. **Zhao C**. Limitations in membrane protein structure determination by lipid nanodiscs. ***Trends Biochem Sci***. 26:S0968-0004(24)00076-8. **2024**. PMID: 38538407

3. Structural and functional characterizations of human K_{ATP} channel.

K_{ATP} channel is a metabolic sensor that translates cellular ATP/ADP ratio into membrane excitability. In pancreatic β cells, K_{ATP} channel closes its pore in response to increased blood sugar levels to regulate insulin secretion. In my work, I determined the first structure of a K_{ATP} channel with an open pore using cryo-EM. The structures revealed allosteric coupling between the pore and the inhibitory ATP binding site. These structural observations offer novel insights into developing anti-diabetic drugs targeting K_{ATP}. In addition, I, for the first time, reconstituted purified K_{ATP} channels into synthetic lipid membranes and discovered that signaling lipid PIP_2 is not obligatory for channel opening. This independence on PIP_2 challenges the common belief in the field and establishes a new framework for understanding K_{ATP} regulation in the cell membrane.

a. **Zhao C** and MacKinnon R. Molecular structure of an open human KATP channel. ***PNAS***. 118 (48) e2112267118. **2021**. PMCID: PMC8640745

4. Structural and functional characterization of group II introns and their encoded maturase proteins.

Group II introns are a class of self-splicing RNAs in prokaryotes and eubacteria. In order to carry out the splicing reaction, group II introns fold into intricate tertiary structures. The catalytic activity of the self-splicing reaction is low, and to boost the reaction rate, group II intron encodes a helper protein called maturase that interacts with intron RNA and facilitates splicing. Besides promoting intron splicing, maturase is also a reverse transcriptase that synthesizes DNA molecules using intron RNA as a template. The product DNA can integrate into the genome, making group II introns "selfish genes." During my Ph.D. training with Dr. Anna Marie Pyle, I have made three major contributions to the field of group II introns. First, I determined the crystal structure of a group II intron folding intermediate and found that RNA folding follows a "first comes, first folds" rule. Second, I determined the first crystal structures of group II intron maturase reverse transcriptase domains and revealed their structural similarities to the eukaryotic spliceosome. Finally, I have discovered a group II intron maturase, as a reverse transcriptase, possesses superior processivity on long and structured RNA templates. This group II intron maturase has now been commercialized as a molecular biology tool to synthesize cDNAs from long and complex RNA templates.

a. **Zhao C**, Rajashankar K.R., Marcia M and Pyle AM. Crystal structure of group II intron domain 1 reveals a template for RNA assembly. ***Nat. Chem. Biol.*** 11, 967-972. **2015.** PMCID: PMC4651773

b. **Zhao C** and Pyle AM. Crystal structures of a group II intron maturase reveal a missing link in spliceosome evolution. ***Nat. Struct. Mol. Biol.*** 23, 558-565. **2016.** PMCID: PMC4899126

c. **Zhao C** and Pyle AM. Structural Insights into the Mechanism of Group II Intron Splicing. ***Trends. Biochem. Sci.*** 42, 470-482. **2017.** PMCID: PMC5492998

d. **Zhao C**, Liu F and Pyle AM. An ultra-processive, accurate reverse transcriptase encoded by a metazoan group II intron. ***RNA***. 24, 183-195. **2018.** PMCID: PMC5769746

My Bibliography Link:

https://www.ncbi.nlm.nih.gov/myncbi/chen.zhao.10/bibliography/public/