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	BS	07/2012	Biological Sciences
Yale University	PhD	12/2017	Molecular Biophysics and Biochemistry
The Rockefeller University	Postdoc	07/2023	Biochemistry, Structural Biology and Physiology

A. Personal Statement

My long-term career goal is to decipher how membrane receptors and channels govern signal transduction pathways involved in neurological processes. My research team harnesses structural biology techniques such as cryo-electron microscopy (cryo-EM), with complementary biochemistry, cell physiology and cell biology techniques, to elucidate the molecular mechanisms underlying the membrane receptors and channels involved in signaling pathways. We will leverage the mechanistic knowledge to facilitate developing innovative therapeutic tools targeting these membrane proteins.

My scientific training has equipped me to effectively lead research projects focused on achieving my long-term goal. During my postdoc training with Dr. Roderick MacKinnon, I characterized the structures and functions of six different membrane proteins by cryo-EM and electrophysiology (three of them are published). Most importantly, I conceptualized the idea of a project that addresses a fundamental limitation in the field: conventional membrane protein structures are evaluated in detergent environments that are substantially different from their native cell membrane environment. In this project, we developed a novel method that enables determining high-resolution membrane protein structures in cell membrane environment via cell membrane-derived vesicles. In my recently established research group, we will leverage and expand this novel method and apply it to a wide variety of receptor and channel proteins implicated in signal transduction in the nervous system.

In addition to research training, 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 to present in 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 two postbac students (one just arrived), two undergraduate students and co-mentoring a postdoc with Dr. Michelle L. Gumz. I will have one Master’s student and a graduate student for summer rotation in the coming semester. With these personnel, my research group is composed of a highly diverse population in terms of gender, race, geographic location, and social-economical background. I have been working with one of the postbac students for 7 months. For this student, I design daily plans and work with him side-by-side. After three months of training, this student is already capable of independently carrying out all basic techniques in the lab including insect and mammalian cell culture, membrane protein purification and cryo-EM data processing. I also have weekly meetings with this student to improve his

scientific knowledge, critical thinking and writing skills. Besides mentoring in the lab, I serve on the supervisory committee of 2 graduate students in the Department of Chemistry, and 1 Master's student in the Department of Biochemistry & Molecular Biology at the University of Florida.

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

Honors

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 the 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 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 through several aspects. First, using cryo-EM, I determined the first and only structure of 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 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 (eg. 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 visualization of a large number of 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 important for membrane protein function. 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 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 reverse transcribe 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. The group II intron maturase: a reverse transcriptase and splicing factor go hand in hand. ***Curr. Opin. Struct. Biol.*** 47, 30-39. **2017.** PMCID: PMC5694389

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

e. **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

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