BIOGRAPHICAL SKETCH

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NAME: Zhao, Yongxiang

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

POSITION TITLE: Postdoctoral Fellow

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	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Wuhan University of Technology, China	BS	09/2007	07/2011	Biotechnology
Wuhan University of Technology, China	MS	09/2011	07/2014	Analytical Chemistry
Wuhan Institute of Physics and Mathematics, China	Ph.D	09/2015	12/2018	Physical Chemistry
University of Utah, United States	Postdoc	07/2019	Present	Biochemistry

A. Personal Statement

As a Ph.D. student with Jun Yang at Wuhan Institute of Physics and Mathematics, Chinese Academy of Science, I was trained as a biochemist and biophysicist, and analyzed the structures and dynamics of Aquaporin Z in lipid bilayers and native cell membrane by MAS solid-state NMR. I developed a strong interest in the molecular mechanisms of membrane channels and transporters, especially those implicated in human health and diseases. I joined Erhu Cao's lab as a postdoctoral fellow at the University of Utah and is training in structure determination by single-particle electron cryo-microscopy (cryo-EM) and physiological/biophysical analyses of human transporters. Currently, I focus on structures, pharmacology, and physiological regulation of cation-chloride cotransporters (CCCs), which play pivotal roles in ion reabsorption in the kidney and chloride homeostasis in neuronal cells. We recently determined several high-resolution cryo-EM structures of human CCCs in different conformational states and in complex with small molecule inhibitors, which provide the initial insights into transport cycle and inhibitory mechanism of CCCs. I am also determining structures of CCCs in phosphorylated state and bound with diuretics so as to understand phosphoregulation of CCCs and modes of action of anti-hypertensive diuretics. My goal is to launch an independent academical career and contribute to the research centered around the structural mechanisms of ion channel and transporters in human health and diseases, especially those implicated in renal physiology and diseases.

Zhao Y, Shen J, Wang Q, Ruiz Munevar MJ, Vidossich P, De Vivo M, Zhou M, Cao E*. Structure of the human cation-chloride cotransport KCC1 in an outward-open state. ***Proc Natl Acad Sci U S A***. 2022 Jul 5;119(27): e2109083119. doi: 10.1073/pnas.2109083119. Epub 2022 Jun 27. PMID: 35759661; PMCID: PMC9271165. * Corresponding authors

Zhao Y, Roy K, Vidossich P, Cancedda L, De Vivo M, Forbush B*, Cao E*. Structural basis for inhibition of the Cation-chloride cotransporter NKCC1 by the diuretic drug bumetanide. ***Nat Commun***. 2022 May 18;13(1):2747. doi: 10.1038/s41467-022-30407-3. PMID: 35585053; PMCID: PMC9117670. * Corresponding authors

Zhao Y[#], Xie H[#], Wang L, Shen Y, Chen W, Song B, Zhang Z, Zheng A, Lin Q, Fu R, Wang J[*], Yang J*. Gating Mechanism of Aquaporin Z in Synthetic Bilayers and Native Membranes Revealed by Solid-State NMR Spectroscopy. ***J Am Chem Soc***. 2018 Jun 27;140(25):7885-7895. doi: 10.1021/jacs.8b03446. PMID: 29799200. [#] Co-first authors * Corresponding authors

Tan H[#], **Zhao Y**[#], Zhao W, Xie H, Chen Y, Tong Q, Yang J*. Dynamics properties of membrane proteins in native cell membranes revealed by solid-state NMR spectroscopy. ***Biochim Biophys Acta Biomembr***. 2022 Feb 1;1864(1):183791. doi: 10.1016/j.bbamem.2021.183791. Epub 2021 Oct 6. PMID: 34624277. [#] Co-first authors * Corresponding authors

B. Positions and Honors

Positions and Employment

2011-2014 Visiting student, Dr. Jun Yang's lab, Wuhan Institute of Physics and Mathematics, China

2014-2015 Research Assistant, Dr. Jun Yang's lab, Wuhan Institute of Physics and Mathematics, China.

2015-2018 Graduate Student, Dr. Jun Yang's lab, Wuhan Institute of Physics and Mathematics, China.

2019-present Postdoctoral Fellow, Dr. Erhu Cao's lab, Department of Biochemistry, University of Utah.

Honors and Awards

2015 /2017 Outstanding student in University of Chinese Academy of Science.

2018 Outstanding graduate in University of Chinese Academy of Science.

2018 Chinese Academy of Science President Award (The Highest Award of Chinese Academy of Science to graduate students).

Professional Membership

American Heart Association, since 2020

American Society of Nephrology, since 2020

C. Contributions to Science

1. **Ph.D. Graduate Studies – Structure, Gating, and Mercury Inhibitory Mechanisms of Aquaporin *Z* in Native Membrane Revealed by Solid-State NMR.**

Aquaporins are water channel in cell membrane essential for water homeostasis and are associated with a number of human diseases. The X-ray crystal structures of Aquaporins in detergent micelles, reported since 2000, have shed light on the molecular mechanisms of water transport and ion selectivity of these membrane proteins. However, little is known about structures and dynamics of Aquaporin in lipid bilayer, especially in native cell membrane. During my Ph.D. study, I optimized the sample of isotopically labeled Aquaporin Z in *E. coli* inner membrane and elucidated the gating, mercury inhibitory and lipid regulatory mechanisms of Aquaporin Z in native cell membrane by solid-state NMR. We determined the first structure of Aquaporin Z in *E. coli* inner membrane, and found that its diameter of water pore is 0.6 Å larger than that reported in X-ray crystal structures through subtle changes of the transmembrane helix orientation, which may provide a novel regulatory mechanism of Aquaporin (recently submitted to *Nature Methods*). We further explored the conformation of R189, a key residue that forms the narrowest constriction in selective filter region of water channel and found that R189 stays in the permanent "up"/open state in native membrane, which disputes the dominant hypothesis that R189 can act as a steric gate in water channel through switching from "up"/open conformation to "down"/close

conformation as reported in the crystal structure and molecular dynamics simulation. The Aquaporin is inhibited by Hg^{2+}, however, molecular mechanism of mercury inhibition is unclear since wildtype Aquaporin is difficult to form ordered crystal for structural analysis when bound with Hg^{2+}. We studied the structure and dynamics of Aquaporin Z bound with Hg^{2+} and found that Hg^{2+} denatures the water channel when binds to Cys20 close to R189 in the selective filter region, and blocks water transport when binds to L170C residue in the central area of water channel, providing novel insights into mercury inhibition of Aquaporin (in revision at *PNAS*).

Zhao Y[#], Xie H[#], Wang L, Shen Y, Chen W, Song B, Zhang Z, Zheng A, Lin Q, Fu R, Wang J[*], Yang J[*]. Gating Mechanism of Aquaporin Z in Synthetic Bilayers and Native Membranes Revealed by Solid-State NMR Spectroscopy. ***J Am Chem Soc***. 2018 Jun 27;140(25):7885-7895. doi: 10.1021/jacs.8b03446. PMID: 29799200. [#] Co-first authors [*] Corresponding authors

Xie H[#], **Zhao Y**[#], Wang J, Zhang Z, Yang J*. Solid-state NMR chemical shift assignments of aquaporin Z in lipid bilayers. ***Biomol NMR Assign***. 2018 Oct;12(2):323-328. doi: 10.1007/s12104-018-9832-5. PMID: 29943128. [#] Co-first authors * Corresponding author

Tan H[#], **Zhao Y**[#], Zhao W, Xie H, Chen Y, Tong Q, Yang J*. Dynamics properties of membrane proteins in native cell membranes revealed by solid-state NMR spectroscopy. ***Biochim Biophys Acta Biomembr***. 2022 Feb 1;1864(1):183791. doi: 10.1016/j.bbamem.2021.183791. Epub 2021 Oct 6. PMID: 34624277. [#] Co-first authors * Corresponding authors

Huayong Xie[#], **Yongxiang Zhao**[#], Weijing Zhao, Yanke Chen, Maili Liu, Jun Yang*. Structure Determination of a Membrane Protein in Native Cellular Membranes. (Under review, ***Nature Methods***). [#] Co-first authors * Corresponding author

2. Postdoctoral Studies – Structures, Physiology, and Pharmacological Regulation of CCCs.

Cation-chloride cotransporters (CCCs) mediate the electroneutral transport of chloride together with sodium and/or potassium across cell membrane driven by the ion gradient created by sodium/potassium ATPases. They play pivotal roles in regulating cell volume, controlling ion absorption and secretion across epithelia, and maintaining intracellular chloride homeostasis. Malfunction or dysregulation of these transporters cause many human disorders, such as hypertension, fluid overload, kidney filtration problems, and epilepsy. These transporters are primary targets of some of the most commonly prescribed loop and thiazide diuretics for the treatment of hypertension and edema. In Dr. Cao lab, I tried to understand the molecular mechanisms whereby CCCs orchestrate conformational changes along their transport cycle, (de)phosphorylation impacts ion translocation pathway, and diuretics interact with and inhibit ion transport by means of cryo-EM and ion influx assay in cells and liposomes. Recently, we determined cryo-EM structures of human KCC1 in both apo inward-open conformation state and outward-open conformation stabilized by the VU0463271 inhibitor, providing initial insights into the critical transport-associated conformational changes in CCCs. The structure of human KCC1-VU0463271 complex provides the molecular details of inhibitor binding with CCCs, and will facilitate rational drug design of CCCs in the future. I also determined a 2.7 Å cryo-EM structure of Drosophila KCC in an inward-facing and auto-inhibited state, in which an auto-inhibitory N-terminal segment binds to and occludes the intracellular ion transport pathway. Recently, we also determined the cryo-EM structure of human NKCC1 bound with bumetanide in a novel dimeric form, in which the transmembrane domains that house ion translocation pathway are dissociated from each other. I am also determining structures of CCCs bound with other anti-hypertensive loop diuretics and phosphoregulation of CCCs, which are the focus of this application.

Zhao Y, Shen J, Wang Q, Ruiz Munevar MJ, Vidossich P, De Vivo M, Zhou M, Cao E*****. Structure of the human cation-chloride cotransport KCC1 in an outward-open state. ***Proc Natl Acad Sci U S A***. 2022 Jul 5;119(27): e2109083119. doi: 10.1073/pnas.2109083119. Epub 2022 Jun 27. PMID: 35759661; PMCID: PMC9271165. * Corresponding authors

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Yongxiang Zhao* and Erhu Cao*. Structural pharmacology of cation-chloride cotransporters. (Invited Review Article for ***Membranes***, under review). * Corresponding authors

D. Additional Information: Research Support and/or Scholastic Performance

2011-2014_ M.S. Courses_ Wuhan University of Technology

YEAR	COURSE TITLE	GRADE
2011-2012	Analytic Chemistry	92/100
2011-2012	Physical Chemistry	90/100
2012-2013	Biochemistry	90/100

2015-2018_ Ph.D. Courses _Wuhan Institute of Physics and Mathematics

YEAR	COURSE TITLE	GRADE
2015-2016	Modern NMR Techniques	89/100
2016-2017	Advanced Biochemistry	88/100
2017-2018	Liquid Nuclear Magnetic Resonance Experimental Techniques	90/100