BIOGRAPHICAL SKETCH

NAME: Zhao, Yongxiang

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

POSITION TITLE: Postdoc 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 *(if applicable)*	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. To obtain broad academical training, I joined Erhu Cao's lab as a Postdoc 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 the structures, pharmacology, and physiological regulation of cation-chloride cotransporters, which play pivotal roles in ion reabsorption in the kidneys and chloride homeostasis in neuronal cells. We recently determined several high resolution cryo-EM structures of human KCC1 in different conformational states and in complex with a small molecule inhibitor, which provide the initial insights into transport cycle and inhibitory mechanism of cation chloride cotransporters. I am also determining structures of cation chloride cotransporter in phosphorylated state and bound with diuretics so as to understand phosphoregulation of cation chloride cotransporters 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[#], 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

Xiao, H., Zhang, Z*., **Zhao, Y**. & Yang, J*. Spectral editing of alanine, serine, and threonine in uniformly labeled proteins based on frequency-selective homonuclear recoupling in solid-state NMR. *J Biomol NMR*.2021 Apr 22; 75, 193-202, doi:10.1007/s10858-021-00367-9. PMID: 33890210. * Corresponding authors

Tong Q, Tan H, Li J, Xie H, **Zhao Y**, Chen Y, Yang J*. Extensively sparse $^{(13)}$C labeling to simplify solid-state NMR (13)C spectra of membrane proteins. *J Biomol NMR*.2021 Jun 20; 75, 245-254, doi:10.1007/s10858-021-00372-y. PMID: 34148188. * 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. *Biochimica et Biophysica Acta (BBA) – Biomembranes*. 2022 Feb 1;1864(1):183791. doi: 10.1016/j.bbamem.2021.183791 [#] Co-first authors * Corresponding author

Yongxiang Zhao, Jiemin Shen, Qinzhe Wang, Ming Zhou, Erhu Cao*. 2020. Inhibitory and Transport Mechanisms of the Human Cation-Chloride Cotransport KCC1. bioRxiv. doi: https://doi.org/10.1101/2020.07.26.221770. * Corresponding authors. (Under second-round review, *PNAS*) * Corresponding author

Yongxiang Zhao, Kasturi Roy, Pietro Vidossich, Laura Cancedda, Marco De Vivo, Biff Forbush*, and Erhu Cao*. Structural Basis for Inhibition of the Cation-chloride Cotransporter NKCC1 by the Diuretic Drug Bumetanide. (Under second-round review, *Nature Communication*) * Corresponding author

Huayong Xie[#], Shaojie Ma[#], **Yongxiang Zhao[#],** Hu Zhou, Qiong Tong, Yanke Chen*, Zhengfeng Zhang, Kunqian Yu*, Qingsong Lin*, Lei Kai, Maili Liu, Jun Yang*. Molecular Mechanisms of Mercury-Sensitive Aquaporins. (Under review, *PNAS*) [#] Co-first authors * Corresponding author

Yan Zhang[#], **Yongxiang Zhao[#]**, Xuning Zhang, Yanke Chen, Qiong Tong*, Jun Yang*. Solid-state NMR ^{13}C and ^{15}N resonance assignment of *Vibrio sp.* SemiSWEET transporter in lipid bilayers. (Under review, *Biomol NMR Assign*) [#] Co-first authors * Corresponding author

B. Positions and Honors

Positions and Employment

2011-2014 Visiting Graduate 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 /2017/2018 Outstanding student 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 membrane, especially in native cell membrane. During my PhD 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 (unpublished data). 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 the 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 (unpublished data).

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

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Tong Q, Tan H, Li J, Xie H, **Zhao Y**, Chen Y, Yang J*. Extensively sparse $^{(13)}$C labeling to simplify solid-state NMR (13)C spectra of membrane proteins. *J Biomol NMR*.2021 Jun 20; 75, 245-254, doi:10.1007/s10858-021-00372-y. PMID: 34148188. * 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. *Biochimica et Biophysica Acta (BBA) – Biomembranes*. 2022 Feb 1;1864(1):183791. doi: 10.1016/j.bbamem.2021.183791 [#] Co-first authors * Corresponding author

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2. **Postdoctoral Studies – Structural and Functional Insights into the Mechanisms of Cation Chloride Cotransporters of Transport Cycle**, **Phosphoregulation and Inhibition by Diuretics.**

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. When I joined Erhu' lab, I tried to understand the molecular mechanisms whereby CCCs orchestrate conformational changes along a 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 liposomes. Recently, we determined cryo-EM structures of human KCC1 in both apo inward-open conformation state and outward-open conformation stabilize by the VU0463271 inhibitor, providing initial insights into 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 inward-facing and auto-inhibited state, in which an auto-inhibitory N-terminal segment binds to and occludes the intracellular ion transport pathway. I am also determining the cryo-EM structures of plant CCCs, which is the focus of this application.

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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