BIOGRAPHICAL SKETCH

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NAME: Hou, Xiaowei

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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
Anhui University	BS	07/2003	Chemical Engineering
University of Science and Technology of China; jointly studied at the Institute of Biophysics, Chinese Academy of Sciences	PHD	07/2008	Molecular Biology and Biochemistry; Structural Biology
Memorial Sloan Kettering Cancer Center	Postdoc, Senior Research Scientist	07/2022	Structural Biology

A. Personal Statement

I am a new Assistant Professor that was recently **appointed in July 2022** to establish an independent research program at the University of Cincinnati College of Medicine in the Department of Molecular and Cellular Biosciences. I have worked on the Ca^{2+} release-activated Ca^{2+} (CRAC) channels since the beginning of my postdoctoral research in Dr. Stephen Long's Lab in the Structural Biology Program at the Memorial Sloan Kettering Cancer Center (MSKCC). Previously, I have determined crystal and cryo-electron microscopy (cryo-EM) structures of Orai, the plasma membrane pore-forming component of CRAC channels, in a *closed*, an *intermediate*, and an *open* conformation. These structures provide unprecedented insights into Ca^{2+} permeation and conformational transitions of the Orai channel toward opening. The long-term goal of my independent research program is to study the molecular regulation of the CRAC channel signaling pathway to uncover the molecular mechanisms underlying the diverse physiological and pathophysiological functions of CRAC channels. Initially, the research program focuses on structural and functional studies on the core complex of CRAC channels, formed by the Orai channels and STIM proteins as the ER Ca^{2+} sensor, as well as interactions between Orai and/or STIM with cellular regulatory factors. During my postdoctoral research, I have developed monoclonal antibody tools and biochemical, biophysical, and liposome/cell based functional assays to study CRAC channel structure and function, which have prepared me well for tackling next key questions regarding the molecular regulation of the CRAC channels. In addition, I had considerable experience in teaching and mentoring. I have independently trained two research technicians, who have published with me and moved forward to pursue their careers in medicine. I have also trained four graduate students, who are making great progress in their research. My strong commitment to both research and mentoring has provided me with vast experience in training the next generation of scientists. Furthermore, I have built tight connections with prominent scientists in the CRAC channel field who can provide support and mentoring in functional and disease studies. The local structural biology community at the University of Cincinnati College of Medicine and Cincinnati Children's Hospital also provides me with strong support and mentoring. Together, my experiences in mentoring, in combination with my expertise in ion channel structure-function studies and strong support from colleagues, have optimally positioned me for success in establishing a broad multidisciplinary research program that is capable of using innovative new technologies and approaches. The work proposed here stems from my momentum and expertise to expand our mechanistic understanding of the molecular regulation of the CRAC channel signaling pathway using a combination of cell biology, biochemistry, biophysics, and structural biology. Answering the basic biological questions surrounding the CRAC channel structure, function, and molecular regulation in human cells will provide an avenue for therapeutic development in treating CRAC channel-associated diseases, which has profound importance in human health.

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B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

07/2022-present	Assistant Professor	University of Cincinnati College of Medicine
2020-present	Member	Society of General Physiologists
2016-2022	Senior Research Scientist	Memorial Sloan Kettering Cancer Center
June-December 2015	Visiting Scientist	HHMI's Janelia Research Campus
2013-2016	Research Associate	Memorial Sloan Kettering Cancer Center
2011-2013	Research Scholar	Memorial Sloan Kettering Cancer Center
2008-2011	Research Fellow	Memorial Sloan Kettering Cancer Center

Honors

2014	Blavatnik Regional Award Finalist in Chemistry
2014	MSKCC Postdoctoral Researcher Award

C. Contributions to Science

1. In the early days of my PhD studies, genome sequences of several organisms were just available, making it possible to answer many appealing biological questions by determining the structures of the gene products. I worked on a few different proteins from pathogenic bacteria to humans, including a SAM-dependent O-methyltransferase from pathogenic bacterium *Leptospira interrogans* (LiOMT) and human mitochondrial outer membrane protein mitoNEET, and received extensive training in molecular biology, biochemistry, and structural biology (X-ray crystallography). (a) LiOMT belongs to a protein family with all known members involved in antibiotic production. The purified LiOMT protein forms a dimer in solution and in the 2.3 Å crystal structure, in which two LiOMTs dimerize through N-terminal swapping. This dimerization appears to be in a pre-organized stable conformation ready for substrate binding. Each monomer has a SAH bound and presents a large empty substrate-binding pocket. Based on the sequence and structural analysis of the catalytic and substrate-binding sites, we propose that the substrate of LiOMT is a phenolic derivative probably with a large ring-shaped moiety. (b) MitoNEET is a single-pass transmembrane protein localized in the outer membrane of mitochondria. It was identified in 2004 as a binding target of the anti-type II diabetes drug pioglitazone and was shown in later studies to regulate the electron transport and oxidative capacity of mitochondria. I focused on the structural study of human mitoNEET in collaboration with Dr. Haining Zhu from the University of Kentucky who are interested in mitochondrial diseases. I determined 1.8 Å crystal structure of mitoNEET. The structure revealed a unique protein fold of mitoNEET dimer with a [2Fe-2S] cluster bound to each monomer. The [2Fe-2S] cluster is coordinated to the protein by novel coordination involving three cysteines and one histidine residue (3Cys1His). The histidine residue might contribute to the special pH sensitivity of the cluster binding. We also proposed that pioglitazone or other proteins might interact with mitoNEET via the surface residues near the [2Fe-2S] cluster and regulate its binding property and redox potential.

    a. **Hou X,** Wang Y, Zhou Z, Bao S, Lin Y, Gong W. Crystal structure of SAM-dependent O-methyltransferase from pathogenic bacterium *Leptospira interrogans*. *J Struct Biol.* 2007 Sep; 159(3):523-8.
    b. **Hou X,** Liu R, Ross S, Smart EJ, Zhu H, Gong W. Crystallographic studies of human mitoNEET. *J Biol Chem.* 2007 Nov 16; 282(46):33242-6.

2. A few later collaborations near the end of my PhD studies drew my attention to membrane proteins that are directly related to physiology and human health and disease. These include a membrane-embedded enzyme diacylglycerol kinase (DGK), the extracellular venus flytrap domains (VFT) of γ-aminobutyric acid (GABA) type B receptor subunit 1 and 2 (GBR1 and 2), and a snake-secreted phospholipasesA2 neurotoxin that inhibits A-type potassium (K^+) currents in rat dorsal root ganglion neurons. As of my PhD graduation, I obtained crystals of DGK that diffracted to ~ 18 Å, succeeded in the expression of the VFT domains of GBRs using the insect cell expression system, and helped in the structural studies of the snake neurotoxin, which resulted in a collaborative publication. From these collaborations, I developed a strong interest in membrane proteins, most of which at the time still lack 3D structural information essential for understanding their important physiological functions. This motivated me to pursue my

postdoc training at the MSKCC to study a family of Ca^{2+} channel proteins that play important roles in human health and disease – the CRAC channels.

a. Hu P*, Sun L*, Zhu ZQ, **Hou X,** Wang S, Yu SS, Wang HL, Zhang P, Wang M, Niu LW, Teng MK, Ruan DY. Crystal structure of Natratoxin, a novel snake secreted phospholipasesA2 neurotoxin from Naja atra Venom inhibiting A-type K^+ currents. *Proteins.* 2008 Aug; 72(2):673-83. (*co-authorship)

3. CRAC channels mediate Ca^{2+} influx across the plasma membrane in response to the depletion of Ca^{2+} stored in the endoplasmic reticulum (ER). The basic mechanism was first described by Jim Putney in 1986 as "capacitive Ca^{2+} entry" or "store-operated Ca^{2+} entry". In the early 90s, electrophysiological recordings of CRAC channels were first measured by whole-cell patch clamp in mast cells and T cells. Defects in CRAC channel function were linked to combined immunodeficiency in patients. In 2005 and 2006, the two molecular components necessary for the CRAC channel function were identified. They are the plasma membrane Ca^{2+} channel Orai and the channel activator STIM, which is localized in the ER membrane. When I started my postdoctoral research in 2008, we knew little about the molecular basis of the CRAC channel's unique electrophysiological properties or the molecular mechanism of the disease-causing mutations identified in the Orai or STIM genes. Since then, I have determined the first crystal structure of *Drosophila* Orai in the *closed* conformation in 2012 (a). This structure revealed unique structural features of Orai, including the hexameric assembly, the selectivity filter, and the unusual chemical environment of the ion-conducting pore. (c) Subsequently, using a gain-of-function mutant of Orai that functions highly similar to wild-type STIM-activated Orai, I determined the structure in an *open* conformation for the first time. Together with the structure of wild-type Orai in an *intermediate* conformation, we proposed a model defining major conformational transitions leading to channel opening. (d) In 2020, using the cryo-EM method and a functionally neutral Fab antibody fragment, I improved the resolution of the *open* channel structure to 3.3 Å, which provides insights into the mechanism of Ca^{2+} permeation through the pore. However, the molecular mechanism of how the CRAC channel is precisely gated and regulated by STIM remains elusive. To answer this question, I have been studying Orai-STIM interaction using a combination of cell biology, biochemistry, biophysics, and structural biology. By far, I have developed functional assays for functional reconstitution of CRAC channels using purified Orai and STIM proteins (reported in b) and obtained a preliminary cryo-EM structure of the Orai-STIM complex. With these functional approaches and a strong background in ion channel biochemistry, biophysics and structural biology, I am thrilled to move forward with my research program to solve the puzzle of CRAC channel regulation and address other fundamental questions in the future.

a. **Hou X,** Pedi L, Diver MM, Long SB. Crystal structure of the calcium release-activated calcium channel Orai. *Science.* 2012 Dec 7; 338(6112):1308-13.
b. **Hou X** and Long SB. Functional reconstitution and structural flexibility of the CRAC channel Orai. *Biophysical Journal.* 2015; 108 (2), 178a. (conference abstract)
c. **Hou X,** Burstein SR, Long SB. Structures reveal opening of the store-operated calcium channel Orai. *Elife*. 2018 Aug 30;7.
d. **Hou X*,** Outhwaite IR*, Pedi L, Long SB. Cryo-EM structure of the calcium release-activated calcium channel Orai in an open conformation. *Elife*. 2020 Nov 30;9. (*co-authorship)

4. I value scientific communication and enjoy reading and writing as well as teaching and mentoring. Between 2017-2018, I wrote a book chapter on ion channels. In this book chapter, I summarized the principal properties of tetrameric cation channels (using K^+ channels as representative) and CRAC channels, with an emphasis on the structures of these channels published within the previous decade.

a. **Hou X.** (2018) Ion Channels. In: Cao Y. (eds) Advances in Membrane Proteins. *Springer, Singapore* (Book chapter)

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/xiaowei.hou.1/bibliography/public/

D. Research Support

Project number	1R35GM151170-01 (Xiaowei Hou)
Source of support	NIH/NIGMS
Title	Molecular regulation of the CRAC channel signaling pathway
Status	Awarded, project period 07/03/2023 – 04/30/2028