

BIOGRAPHICAL SKETCH**DO NOT EXCEED FIVE PAGES**

NAME: Cao, Erhu

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

POSITION TITLE: Associate 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
Huazhong Agricultural University, China	B.S.	07/1996	Plant Biology
Albert Einstein College of Medicine, USA	Ph.D.	03/2007	Biomedical Science
University of California, San Francisco, USA	Postdoctoral	08/2015	Molecular Pharmacology

A. Personal Statement

As a joint Ph.D. student with Steven Almo and Stanley Nathenson at the Albert Einstein College of Medicine, I trained as biochemist and structural biologist, and determined multiple X-ray crystal structures of immunoglobulin family receptors, ligands, and their complexes, including the first structure of the complex between PD-1 and its ligand PD-L2. These studies contributed significantly to our understanding of cellular immunity. As a postdoc in David Julius' laboratory at UCSF, I pursued biochemical, biophysical and structural analyses of transient receptor potential (TRP) ion channels, which are key players in sensory signaling and in the detection of noxious stimuli. My work there included biophysical, pharmacological and structural studies of the TRPV1 ion channel. Consequently, I have extensive experience in molecular biology, protein biochemistry, ion channel reconstitution into liposomes, ion channel electrophysiology, single-particle electron cryo-microscopy (cryo-EM), and protein X-ray crystallography. As an independent faculty member at the University of Utah, my goal is to elucidate the structure, pharmacology, function, and pathophysiology of ion channels, receptors, and transporters that play pivotal roles in renal physiology and which, when mutated, cause kidney diseases in humans. We employ a multidisciplinary approach that includes molecular biology, protein biochemistry, pharmacology, ion channel electrophysiology (patch clamp recording), ion flux assays, X-ray crystallography, and single-particle cryo-EM to achieve a comprehensive and in-depth understanding of sensory and transport mechanisms in the kidneys.

- Yongxiang Zhao, Heidi Schubert, Alan Blakely, Biff Forbush, Micholas Smith, Jesse Rinehart & **Erhu Cao**. Structural Bases for Na⁺-Cl⁻ Cotransporter Inhibition by Thiazide Diuretic Drugs and Activation by Kinases. *Nature Communications*. 2024 Aug 14;15(1):7006. PMCID: PMC11324901
- Yongxiang Zhao & **Erhu Cao**. Structural pharmacology of cation-chloride cotransporters. *Membranes*. 2022, 12, 1206.
- Yongxiang Zhao, Pietro Vidossich, Biff Forbush, Junfeng Ma, Jesse Rinehart, Marco De Vivo, and **Erhu Cao**. Structural Basis for NKCC1 Inhibition by Two Chemical Classes of Loop Diuretic Drugs. *EMBO J*. In press.

Ongoing and completed research projects that I would like to highlight include:

R01 DK127277

Delling/Cao (MPI)

09/15/2021 – 09/15/2025

A comprehensive map of polycystin channel regulation and its implications in polycystic kidney disease

R01 DK128592

Cao (PI)

9/1/2021 – 8/31/2025

Structures and Pharmacology of Cation-chloride cotransporters

1R01DK139541 Cao (PI)

7/1/2024 – 6/30/2029

Structures and Mechanisms of Polycystic Kidney Disease Proteins

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022 – present	Associate Professor, Department of Biochemistry, University of Utah School of Medicine.
2015 - present	Member, American Society of Nephrology
2015 – 2022	Assistant Professor, Department of Biochemistry, University of Utah School of Medicine.
2014 - present	Member, American Heart Association 2010 - present Member, Biophysical Society
2007 – 2015	Postdoctoral Fellow, David Julius Laboratory, Department of Physiology, UCSF.
2001 – 2007	Graduate Student, Steven Almo and Stanley Nathenson laboratories, Albert Einstein College of Medicine.
1999 – 2001	Research Assistant, Youxin Jin lab, Shanghai Institute of Biochemistry, Shanghai, China.

Honors

2017 – 2021	Pew Scholar Award (a highly competitive award that recognizes ~ 20 junior faculty members nationwide each year)
2008 – 2010	Damon Runyon Cancer Research Foundation Postdoctoral Fellowship
2007	Julius Marmur Research Award (Albert Einstein College of Medicine Highest Award given to the best graduate students)

C. Contributions to Science

1. Independent Investigator – structural mechanisms of polycystic kidney disease proteins.

One of our research areas concerns the 11-transmembrane (TM)-spanning PKD1 receptor and tetrameric 6-TM-spanning PKD2 channel, which are sites of mutations that cause the prevalent and life-threatening human genetic disorder autosomal dominant polycystic kidney disease (ADPKD). PKD2 shows distant sequence similarity with the TRPV1 channel whose properties I characterized and structures I determined as a postdoctoral fellow. In 2016, we reported the first cryo-EM structure of the PKD2 channel in a closed state in lipidic nanodiscs at 3.0 Å resolution, which represents the second near-atomic reconstruction of a membrane protein in nanodiscs; the first such technical breakthrough was achieved with TRPV1, whose structures my colleagues and I also determined in nanodiscs (see below). To understand the gating mechanisms (i.e., the process in which a channel opens and closes) of PKD2, we then determined the structure of a gain-of-function PKD2 mutant (F604P) captured in a partially open state, providing initial insights into activation-associated conformational changes in PKD2. To understand pathogenic mechanisms of disease-associated PKD2 variants, we recently reported a structure of PKD2 C331S, demonstrating that this pathogenic variant maintains the tetrameric architecture, but exhibits decreased stability caused by disruption of a conserved disulfide bond formed between C331 and C343. In collaboration with Dr. Paul Decaen at Northwestern University, we showed that C331S and many other PKD2 disease-associated variants show diminished voltage sensitivity, establishing that ADPKD is not only a ciliopathy as widely appreciated, but also a channelopathy in which defects in channel biogenesis and/or gating are the root cause. Most recently, in collaboration with Dr. Markus Delling at UCSF, we showed that the large ectodomain of PKD1 (> 3000 amino acids) functions as an agonist for activation of the PKD1/PKD2 complex, providing initial insights into regulation of this enigmatic receptor/ion channel complex.

- a. Peter S. Shen[#], Xiaoyong Yang[#], Paul G. DeCaen, Xiaowen Liu, David Bulkley, David E. Clapham*, and **Erhu Cao***. The structure of the polycystic kidney disease 2 channel in lipid nanodiscs. *Cell* (2016) 167: 763-773. PMID: PMC6055481 [#] Co-first authors * Corresponding authors
- b. Wang Zheng[#], Xiaoyong Yang[#], Ruikun Hu, Ruiqi Cai, Laura Hofmann, Zhifei Wang, Qiaolin Hu, Xiong Liu, David Bulkley, Yong Yu, Jingfeng Tang*, Veit Flockerzi, Ying Cao, **Erhu Cao***, and Xing-Zhen Chen^{*}. Hydrophobic pore gates regulate ion permeation in polycystic kidney disease 2 and 2L1 channels. *Nature Communications* (2018) 9:2302. PMID: PMC5998024. [#] Co-first authors * Corresponding authors
- c. Thuy N. Vien[#], Jinliang Wang[#], Leo C.T. Ng, **Erhu Cao**, and Paul DeCaen. Molecular dysregulation of ciliary polycystin-2 channels caused by variants in the TOP domain. *Proceedings of the National Academy of Sciences*. 2020 May 12;117(1):2359. PMID: PMC7229662. [#] Co-first authors
- d. Kotdaji Ha, Mai Nobuhara, Qinzhe Wang, Rebecca V Walker, Feng Qian, Christoph Schartner, **Erhu Cao**, Markus Delling. The heteromeric PC-1/PC-2 polycystin complex is activated by the PC-1 N-terminus. *eLife*. 2020 Nov 9;9:e60684. PMID: PMC7728438.
- e. Kodaji Ha, Nadine Mundt-Machado, Paola Bisignano, Aide Pinedo, David R Raleigh, Gabriel Loeb, Jeremy F Reiter, **Erhu Cao**, Markus Delling. Cilia-enriched oxysterol 7 β ,27-DHC is required for polycystin ion channel activation. 2024 Jul 31;15(1):6468. PMID: PMC11291729.

2. Independent Investigator – structures and pharmacology of cation-chloride cotransporters.

More recently, we have started to take a holistic view of the fascinating sensory and transport systems in the kidneys. We were drawn to the cation-chloride cotransporters (CCCs) because two family members (NKCC2 and NCC) are fundamental in salt reabsorption in the kidneys, and, consequently, contribute to maintenance of blood volume and pressure. Several members of the CCC family also play pivotal roles in inhibitory synaptic transmission in the nervous system and are implicated in brain disorders and psychiatric diseases. Pharmacologically targeting CCCs thus represents a promising therapeutic strategy for the treatment of numerous human diseases. Indeed, loop and thiazide diuretics inhibit NKCC2 and NCC, respectively, and are widely prescribed to treat hypertension and edema decades before their molecular targets were finally identified in 1990s. Our contributions in this area so far include: 1) a published structure of human NKCC1 captured in a partially loaded, dephosphorylated (inactive), inward open state; 2) a published KCC1 structure arrested in an outward open state by an inhibitor; and 3) a published human NKCC1 structure bound with bumetanide.

- a. Xiaoyong Yang[#], Qinzhe Wang[#], and **Erhu Cao**. Structure of the human cation-chloride cotransporter NKCC1 determined by single-particle electron cryo-microscopy. *Nature Communications*. 2020 Feb 21;11(1):1016. PMID: PMC7035313. [#] Co-first authors
- b. Zhao, Y., Shen, J., Wang, Q., Zhou, M. & **Erhu Cao**. Structure of the human cation-chloride cotransporter KCC1 in an outward-open state. *PNAS*. 2022 Jul 5; 119(27):e2109083119. PMID: PMC9271165
- c. 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 loop diuretic drug bumetanide. *Nature Communications*. 2022 May 18;13(1):2747. PMID: PMC9117670
- d. Manuel José Ruiz Munevar, Valerio Rizzi Corinne, Portioli Pietro Vidossich, **Erhu Cao**, Michele Parrinello, Laura Cancedda, & Marco De Vivo. Cation Chloride Cotransporter NKCC1 Operates through a Rocking-Bundle Mechanism. *J Am Chem Soc* 2024 Jan 10;146(1):552-566. doi: 10.1021/jacs.3c10258. Epub 2023 Dec 25.

3. Postdoctoral studies – structural and functional insights into the mechanisms of TRPV1 activation and drug action.

My postdoctoral work in the Julius lab at UCSF focused on the structure and physiology of sensory TRP channels, particularly the TRPV1 channel. TRPV1 is a major player in the pain pathway, and is capable of

detecting and integrating a wide range of pain-producing physiological and environmental stimuli. These include noxious heat, proton, pro-inflammatory agents, as well as natural products (e.g., capsaicin from chili pepper and peptide toxins present in spider venoms) that have evolved as defense mechanisms to discourage herbivory or to deter predators. Indeed, elucidating roles of TRPV1 in temperature and pain sensation was honored with the 2021 Nobel Prize in Physiology and Medicine to Dr. David Julius.

In one research direction, I reconstituted purified TRPV1 into giant liposomes and characterized its intrinsic properties by patch clamp recording. By taking this reductionist approach, I showed that TRPV1 is directly activated by heat without the requirement for any other cellular factors. Moreover, I demonstrated that TRPV1 is directly inhibited by PIP₂, thereby providing a mechanistic explanation for how TRPV1 is sensitized by numerous pro-inflammatory agents that lead to phospholipase C (PLC) activation and consequent PIP₂ degradation.

In addition, I collaborated with Maofu Liao in Yifan Cheng' lab at UCSF, and together we determined structures of the TRPV1 channel locked in three distinct functional states (i.e. closed, partially activated, and fully activated) by single particle cryo-EM. These structures revealed a unique two-gate mechanism of channel activation, which includes an unusually dynamic outer pore region, which participates in channel sensitization by tissue acidosis and possibly by other pro-inflammatory agents as well. We also resolved binding sites for pain-producing spider toxins and pungent natural products, and determined how these sites relate to mechanisms of channel activation.

This work represented a seminal achievement in the field because it provided a landmark blueprint for future biophysical and pharmacological studies of TRP channels. Delineation of TRPV1 structure to near atomic resolution without the need to obtain protein crystals also represented a technical breakthrough in single particle cryo-EM. Our success had significant ramifications for the future of membrane protein structure determination because it opened the way for determining receptor and ion channel structures in the many cases where material is limiting or conformationally heterogeneous. Indeed, our TRPV1 structures ushered in a new era of membrane protein structural biology in which membrane protein structures can be routinely determined by single-particle cryo-EM. The summary in the accompanying "News and Views" highlight from Dr. Richard Henderson read: "Structures of the heat-sensitive TRPV1 ion channel have been solved using single-particle electron cryo-microscopy, representing a landmark in the use of this technique for structural biology." My work on TRPV1 contributed to the 2021 Nobel prize in Physiology and Medicine awarded to my postdoc mentor David Julius.

- a. **Erhu Cao**, Julio F. Cordero-Morales, Beiying Liu, Feng Qin, and David Julius. TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids. *Neuron* (2013) February; 77:667-679. PMID: PMC3583019.
- b. ***Erhu Cao**, *Maofu Liao, Yifan Cheng, and David Julius. TRPV1 structures in distinct conformations reveal activation mechanisms. *Nature* (Article with News and Views written by Richard Henderson) (2013) 504:113-118. PMID: PMC4023639. * Equally contributing authors
- c. *Maofu Liao, ***Erhu Cao**, David Julius, and Yifan Cheng. Structure of the TRPV1 channel determined by electron cryo-microscopy. *Nature* (Article with News and Views written by Richard Henderson) (2013) 504:107-112. PMID: PMC4078027. * Equally contributing authors
- d. Yuan Gao, **Erhu Cao**, David Julius, and Yifan Cheng. TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action. *Nature* (2016) 534:347-351. PMID: PMC4911334.

4. Ph.D. graduate studies - structural mechanisms of regulation of cellular immunity by NTB-A, TIM-3 and PD-1 receptors.

NTB-A, TIM-3, and PD-1 all belong to the immunoglobulin (Ig) superfamily of receptors that are capable of stimulating or inhibiting a variety of immune cells (e.g. T cells, B cells, and natural killer cells) upon engaging their cognate ligands via extracellular Ig domains. Notably, tumor cells often exploit the immunosuppressive interactions between PD-1 and its ligands (PD-L1 and PD-L2) to evade immune surveillance. Indeed, antibodies that block the inhibitory PD-1/PD-L1 pathway can boost immune responses against malignant cells in patients, and this advance was honored with the 2018 Nobel Prize in Medicine to Drs. Honjo and Allison. In my graduate work, I determined crystal structures of NTB-A, TIM-3, and PD-1 in complex with PD-L2, and performed

associated biochemical analyses. Together, these findings provide structural blueprints for understanding ligand recognition by these receptors and suggest sites or hot spots that can be targeted for the development of novel immunotherapies to treat cancer and other immune related diseases.

- a. Xuewu Zhang, Jean-Claude D. Schwartz, Xiaoling Guo, Sumeena Bhatia, **Erhu Cao**, Michael Lorenz, Michael Cammer, Lieping Chen, Zhong-Yin Zhang, Michael A. Edidin, Stanley G. Nathenson, and Steven C. Almo. Structural and functional analysis of the costimulatory receptor programmed death-1. *Immunity* (2004) 20: 337-47. PMID: 15030777.
- b. **Erhu Cao**, Udupi A. Ramagopal, Alexander Fedorov, Elena Fedorov, Qingrong Yan, Jeffrey W. Lary, James L. Cole, Stanley G. Nathenson, and Steven C. Almo. NTB-A Receptor Crystal Structure: Insights into Homophilic Interactions in the Signaling Lymphocytic Activation Molecule Receptor Family. *Immunity* (2006) 25: 559-70. PMID: 17045824.
- c. **Erhu Cao***, Xingxing Zang*, Udupi A. Ramagopal*, Arunika Mukhopadhaya, Alexander Fedorov, Elena Fedorov, Wendy D. Zencheck, Jeffrey W. Lary, James L. Cole, Haiteng Deng, Teresa P. DiLorenzo, James P. Allison, Stanley G. Nathenson, and Steven C. Almo. T Cell Immunoglobulin Mucin-3 Crystal Structure Reveals a Galectin-9- independent Ligand-Binding Surface. *Immunity* (2007) 26: 311-321. PMID: 17363302. * Equally contributing authors
- d. *Eszter Lázár-Molnár, *Qingrong Yan, ***Erhu Cao**, Udupi Ramagopal, Stanley G. Nathenson and Steven C. Almo. Crystal structure of the complex between programmed death-1 (PD-1) and its ligand PD-L2. *Proc Natl Acad Sci U S A.* (2008) 105: 10483-8. PMCID: PMC2492495. * Equally contributing authors

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