

**BIOGRAPHICAL SKETCH**  
**DO NOT EXCEED FIVE PAGES.**

NAME: Cao, Erhu

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

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
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 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 of receptors, ligands, or their complexes that contribute to cellular immunity. As a postdoc in the 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. Consequently, I have extensive experience in molecular biology, protein biochemistry, ion channel reconstitution, 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 are implicated in kidney diseases in human. We employ a multidisciplinary approach that includes molecular biology, protein biochemistry, pharmacology, ion channel electrophysiological (patch clamp recording), X-ray crystallography, and single-particle electron cryo-microscopy to achieve a comprehensive and in-depth understanding of sensory and transport mechanisms in the kidneys.

1. Peter S. Shen<sup>#</sup>, Xiaoyong Yang<sup>#</sup>, Paul G. DeCaen, Xiaowen Liu, David Bulkley, David E. Clapham<sup>\*</sup>, and **Erhu Cao**<sup>\*</sup>. The structure of the polycystic kidney disease 2 channel in lipid nanodiscs. *Cell* (2016) 167: 763-773. <sup>#</sup> Co-first authors <sup>\*</sup> Corresponding authors. PMID: PMC6055481
2. Wang Zheng<sup>#</sup>, Xiaoyong Yang<sup>#</sup>, Ruikun Hu, Ruiqi Cai, Laura Hofmann, Zhifei Wang, Qiaolin Hu, Xiong Liu, David Bulkey, Yong Yu, Jingfeng Tang<sup>\*</sup>, Veit Flockerzi, Ying Cao, **Erhu Cao**<sup>\*</sup>, and Xing-Zhen Chen<sup>\*</sup>. Hydrophobic pore gates regulate ion permeation in polycystic kidney disease 2 and 2L1 channels. *Nature Communications* (2018) 9:2302. <sup>#</sup> Co-first authors <sup>\*</sup> Corresponding authors. PMID: PMC5998024.
3. Xiaoyong Yang<sup>#</sup>, Qinzhe Wang<sup>#</sup>, 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. <sup>#</sup> Co-first authors. PMID: PMC7035313.

4. Thuy N. Vien<sup>#</sup>, Jinliang Wang<sup>#</sup>, 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(10):2359. <sup>#</sup> Co-first authors. PMCID: PMC7229662.

## B. Positions and Honors

### Position/Employment

1999 – 2001	Research assistant, Youxin Jin lab, Shanghai Institute of Biochemistry, Shanghai, China.
2001 – 2007	Graduate student, Steven Almo and Stanley Nathenson laboratories, Albert Einstein College of Medicine.
2007 – 2015	Postdoctoral fellow, David Julius Laboratory, Department of Physiology, UCSF.
2015 – present	Assistant professor, Department of Biochemistry, University of Utah School of Medicine.

### Honors

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

### Professional Membership

2010	Biophysical Society, since
2014	American Heart Association
2015	American Society of Nephrology

## C. Contributions to Science

### 1. Independent Investigator – structures and functions of renal sensory and transport proteins.

One of our research area concerns the PKD1 receptor and PKD2 channel, which are sites of mutations that cause the prevalent human genetic disorder autosomal dominant polycystic kidney disease (ADPKD). PKD2 shows distant sequence similarity with the TRPV1 channel whose properties I characterized and structure I determined as a postdoctoral fellow. In 2016, we reported the first cryo-EM structure of the PKD2 channel in closed state in lipidic nanodiscs at 3.0 Å resolution. In order to understand the gating mechanisms (i.e., the process that a channel opens and closes) of PKD2, we 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. In order to understand the pathogenic mechanisms of disease-associated PKD2 variants, we recently reported a structure of PKD2 C331S, demonstrating that disruption of a conserved disulfide bond destabilizes the channel and diminishes voltage sensitivity of the channel.

We are also interested in elucidating structures, physiology, and pharmacology of cation-chloride cotransporters. I was initially fascinated with these transporters 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. Cation-chloride cotransporters also play pivotal roles in inhibitory synaptic transmission in the nervous system and are implicated in brain disorders and psychiatric diseases. Pharmacological targeting cation-chloride transporters 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 cloned in 1990s. Our success in this area so far includes a published structure of human NKCC1 captured in an inward open state and unpublished results detailed in this proposal.

- a. Peter S. Shen<sup>#</sup>, Xiaoyong Yang<sup>#</sup>, 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. <sup>#</sup> Co-first authors \* Corresponding authors. PMCID: PMC6055481

- b. Wang Zheng<sup>#</sup>, Xiaoyong Yang<sup>#</sup>, Ruikun Hu, Ruiqi Cai, Laura Hofmann, Zhifei Wang, Qiaolin Hu, Xiong Liu, David Bulkey, 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. <sup>#</sup> Co-first authors \* Corresponding authors. PMCID: PMC5998024
- c. **Erhu Cao**. Structural mechanisms of transient receptor potential ion channels. *Journal of General Physiology*. 2020 Mar 2;152(3). PMCID: PMC7054860
- d. Xiaoyong Yang<sup>#</sup>, Qinzhe Wang<sup>#</sup>, 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. <sup>#</sup> Co-first authors. PMCID: PMC7035313
- e. Thuy N. Vien<sup>#</sup>, Jinliang Wang<sup>#</sup>, 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(10):2359. <sup>#</sup> Co-first authors. PMCID:PMC7229662

## 2. 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 are currently in clinical trial for treating various cancers. In my graduate work, I have 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 Mukhopadhyaya, 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
- 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

## 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, in particular, the TRPV1 channel. TRPV1 is a major player in the pain pathway, which 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 found in chili pepper and peptide toxins present in spider venoms) that are evolved as defense mechanisms to discourage herbivory or to deter predators.

In one research direction, I reconstituted purified TRPV1 into giant liposomes that allow for characterization of its intrinsic properties by patch clamp recording. By taking this reductionist's 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 PIP<sub>2</sub>, thereby providing a mechanistic explanation for how TRPV1 is sensitized by numerous pro-inflammatory agents that lead to activation of phospholipase C (PLC) and consequent PIP<sub>2</sub> degradation. This approach will be extremely powerful in deciphering the functions of polycystin proteins, which are the focus of this proposal, because they exhibit predominant endoplasmic reticulum localization and hence are not amenable to conventional patch clamp recording at the plasma membrane.

In addition, I collaborated with Maofu Liao in Yifan Cheng's 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, and indicated the relevance of this mechanism to channel sensitization by tissue acidosis and other pro-inflammatory agents. 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 represents 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. I believe that our success has enormous ramifications for the future of membrane protein structure determination because it opens the way to determining receptor and ion channel structures in the many cases where material is limiting or conformationally heterogeneous.

- 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. PMCID: 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. PMCID: 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. PMCID: 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. PMCID: PMC4911334.

#### **Complete List of Published Work in MyBibliography**

<http://www.ncbi.nlm.nih.gov/sites/myncbi/erhu.cao.1/bibliography/48756658/public/?sort=date&direction=ascending>

#### **D. Research Support**

##### **Ongoing Research Support**

R01 DK110575

Cao (PI)

09/01/2016 – 09/01/2021

National Institutes of Health

Structures and Mechanisms of Polycystic Kidney Disease Proteins

The goal of this proposal is to elucidate the structural principles and fundamental biophysical properties of polycystic kidney disease proteins, which are the sites of mutations that cause autosomal dominant polycystic kidney disease. Treatment options for this prevalent genetic disorder are currently limited, in large part because the molecular mechanisms of the relevant proteins are only poorly understood. Successful outcome will provide structural and biochemical insights that will inform the development of novel therapeutic strategies.

Pew Scholar Award  
The Pew Charity Trust  
Title?

Cao (PI)

08/01/2017 – 08/01/2021

This award is meant to support exploratory and highly risky research efforts in the lab.

### **Completed Research Support**

Seed Grant  
University of Utah Research Foundation  
Pharmacology of Polycystic Kidney Disease Proteins  
Goal of the project?

Cao (PI)

07/01/2016 – 06/30/2017

DoD Discovery Grant  
Department of Defense  
Pharmacology of Polycystic Kidney Disease Proteins

Cao (PI)

08/01/2016 – 01/31/2019

The major aim of this project is to develop pharmacological tools (e.g., small chemical compounds, peptide toxins from venomous animals, and conformation-sensitive, functional antibodies) for dissecting structures and physiology of polycystic kidney disease proteins. Such pharmacological studies of PKD proteins will also help to establish therapeutic principles for treating autosomal dominant polycystic kidney disease.