BIOGRAPHICAL SKETCH

NAME: **Youzhong Guo**

eRA COMMONS USER NAME: YZ2271

POSITION TITLE: Assistant Professor of Medicinal Chemistry

INSTITUTION AND LOCATION	DEGREE	Completion Date MM/YYYY	FIELD OF STUDY
The University of Texas at Austin, Austin, TX	Ph.D.	10/2010	Medicinal Chemistry/ Structural Biology
Columbia University, New York, NY	Postdoc	01/2016	Membrane Protein Structural Biology

A. Personal Statement

My research is focused on the structural biology of membrane proteins. I am interested in deciphering the structures and functions of selected biologically or biomedically important membrane proteins and complexes at the atomic-level. While I use comprehensive modern biophysical, biochemical and computational techniques, my main tools are single particle cryo-EM and X-ray crystallography. I am also interested in novel methods development for membrane protein structural biology. I have developed an efficient and economic lipidic cubic phase method for crystallization and an iodine phasing method for structural determination of membrane proteins with X-ray crystallography. These methods have resulted in structural determinations of eight unique membrane proteins. I have been also developing a novel native cell membrane nanoparticles system for high-resolution structural determination for single particle cryo-EM. With this method, I have recently determined the structure for a multidrug transporter complexed with its native membrane lipid bilayer, and discovered that lipid molecules self-organize in a hexagonal pattern in the lipid bilayer. This is the first time that the well-organized high-resolution structure of lipid bilayer has directly been observed with single particle cryo-EM. This is a revolutionary development for membrane protein structural biology, and will likely have a profound effect on both biology and drug discovery and development.

1. Weihua Qiu, Ziao Fu, Guoyan G. Xu, Robert A. Grassucci, Yan Zhang, Joachim Frank, Wayne A. Hendrickson, **Youzhong Guo**. (2018) Structure and Activity of Lipid Bilayer within a Membrane Protein Transporter. *Proc. Natl. Acad. Sci. USA* **115**: 12985-12990.
2. Min Su, Yange Mao, Qi Yuan, Feng Gao, De-lin Li, **Youzhong Guo**, Cheng Yang, Xiao-hui Wang, Renato Bruni, Brian Kloss, Hong Zhao, Yang Zeng, Fa-ben Zhang, and Andrew Marks Wayne Hendrickson, Yu-hang Chen.(2017) Structural basis for conductance through TRIC cation channels. *Nature Communication*. (Article number: 15103 (2017)
3. **Youzhong Guo**, Ravi C. Kalathur, Qun Liu, Brian Kloss, Renato Bruni, Christopher Ginter, Edda Kloppmann, Burkhard Rost and Wayne A. Hendrickson. (2015) Structure and activity of tryptophan- rich TSPO proteins. *Science* **347**: 551-554.
4. Tingting Yang, Qun Liu, Brian Kloss, Renato Bruni, Ravi C. Kalathur, **Youzhong Guo**, Edda Kloppmann, Burkhard Rost, Henry M. Colecraft and Wayne A. Hendrickson. (2014) Structure and selectivity in bestrophin ion channels. *Science* **346**: 355-359.

B. Positions and Honors

Positions and Employment

2016-Present **Assistant Professor:** Institute for Structural Biology, Drug Discovery and Development (ISBDDD), Depart. of Medicinal Chemistry, School of Pharmacy (SOP), VCU

Honors

2000 Di Ao Scholarship, Academy of Sciences P. R. China

C. Contribution to Science

1. Native cell membrane nanoparticles system for cryo-EM: More than 50% of modern drugs target human membrane proteins. High-resolution structures of human membrane proteins in their native and functional states are in high-demand. However, to date, only a few of such structures have been reported and almost all of them are heavily engineered and extracted with various detergents. Although the lipid bilayer is the native environment of membrane proteins, detergents always destroy it and thus membrane protein structures determined in this way may not be biologically relevant. One typical representative is in structure determination of GPCRs, where the third loop is often deleted and a T4 lysozyme or another protein has been inserted for stabilization and crystallization. In contrast, single particle cryo-EM has recently emerged as a very powerful method for structure determination of membrane proteins and complexes. Cryo-EM is also currently beset with problems and challenges. I have been developing a novel native cell membrane nanoparticles system for single particle cryo-EM structure determination of these proteins using single particle cryo-EM since I arrived at VCU. It has shown to be successful in a structural determination of a multiple drug transporter. In fact, we observed a high-resolution structure of the lipid bilayer itself, which we believe to be a first.

1. Weihua Qiu, Ziao Fu, Guoyan G. Xu, Robert A. Grassucci, Yan Zhang, Joachim Frank, Wayne A. Hendrickson, **Youzhong Guo**. (2018) Structure and Activity of Lipid Bilayer within a Membrane Protein Transporter. *Proc. Natl. Acad. Sci. USA* **115**: 12985-12990.

2. Structure and activity of tryptophan-rich sensory protein (TSPO): Valium and other benzodiazepine drugs are well-known prescription drugs in the United States. Valium targets the $GABA_A$ receptor as well as the translocation protein TSPO. However, for about four decades since its discovery as a PBR receptor, the structure and real function of TSPO remained elusive. TSPO is a membrane protein located on the mitochondria and such proteins are very challenging to study. While working with Dr. Wayne A. Hendrickson as a postdoctoral at Columbia University, I successfully solved multiple high-resolution crystal structures of a TSPO protein including a TSPO/PK11195 complex, where [^{11}C] PK11195 is a well-known Positron Emission Topography (PET) probe specifically targeting TSPO. This technique has been used for diagnosing neuroninflammation and related diseases such as Alzheimer's diseases. Also notable is that I discovered that TSPO is a novel enzyme in that it degrades protoporphyrin IX into bilindigin. This work resulted a publication in Science. Furthermore, the new understanding of TSPO provides a solid basis for structure-based drug design and elucidating the molecular mechanism and biological role of TSPO in many physiological and pathological conditions.

1. **Youzhong Guo**, Ravi C. Kalathur, Qun Liu, Brian Kloss, Renato Bruni, Christopher Ginter, Edda Kloppmann, Burkhard Rost and Wayne A. Hendrickson. (2015) Structure and activity of tryptophan- rich TSPO proteins. *Science* **347**: 551-554.

3. High-resolution structure determination with X-ray crystallography: High-resolution structures of membrane proteins have been rare. X-ray crystallography is a predominant method for structure determination of proteins. At Columbia University and now as an Assistant Professor at VCU, I have been developing new, efficient and economical LCP methods for X-ray crystallographic structural determination of membrane proteins. The lipid cubic phase (LCP) mimics a membrane protein bilayer, and thus provides a more comfortable environment for membrane protein; however, commercial LCP kits are both costly and inefficient. My development is a novel adjustable metal LCP syringe coupler and efficient protocols for large-scale crystallization screens of membrane proteins in LCP. With my protocol, eight unique membrane proteins haven crystallized in LCP. Another problem in determination of novel crystal structures is *phasing*, and I also developed a novel and very robust iodine phasing method for crystallographic structure determination of membrane proteins. This development has resulted in four publications at present.

1. Min Su, Yange Mao, Qi Yuan, Feng Gao, De-lin Li, **Youzhong Guo**, Cheng Yang, Xiao-hui Wang, Renato Bruni, Brian Kloss, Hong Zhao, Yang Zeng, Fa-ben Zhang, and Andrew Marks Wayne Hendrickson, Yu-hang Chen.(2017) Structural basis for conductance through TRIC cation channels. *Nature Communication*. (paper #NCOMMS-16-23951B, accepted)

2. **Youzhong Guo**, Ravi C. Kalathur, Qun Liu, Brian Kloss, Renato Bruni, Christopher Ginter, Edda Kloppmann, Burkhard Rost and Wayne A. Hendrickson. (2015) Structure and activity of tryptophan- rich TSPO proteins. *Science* **347**: 551-554.
3. Tingting Yang, Qun Liu, Brian Kloss, Renato Bruni, Ravi C. Kalathur, **Youzhong Guo**, Edda Kloppmann, Burkhard Rost, Henry M. Colecraft and Wayne A. Hendrickson. (2014) Structure and selectivity in bestrophin ion channels. *Science* **346**: 355-359.
4. Qun Liu, **Youzhong Guo**, Yanqi Chang, Zheng Cai, Zahra Assur, Filippo Mancia, Mark I. Greened and Wayne A. Hendrickson. (2014) Multi-crystal native SAD analysis at 6 keV. *Acta Crystallogr D Biol Crystallogr.* **70**: 2544-2557.

4. Structural enzymology: Organic chloride pollution in soils is a big problem for agriculture in the United States. At the University of Texas (Austin), while working with Dr. Christian P. Whitman and Dr. Marvin L. Hackert as a Ph. D student, my research focused on the structural enzymology of the tautomerase superfamily. Enzymes within this superfamily are responsible for degradation of organic chlorides. During that period, I successfully solved and deposited more than 10 crystal structures for four distinct proteins into the Protein Data Bank, including two articles as first author. My work revealed how a general β-α-β motif serves as a building block that yields versatile enzymes with similar overall structures. The basic research may gradually lead to some engineered soil bacteria for clearing organic chloride polluted soils.

1. **Youzhong Guo**, Hector Serrano, Gerrit J. Poelarends, William H, Johnson, Jr., Marvin L. Hackert, Christian P. Whitman. (2013) Kinetic, Mutational, and Structural Analysis of Malonate Semialdehyde Decarboxylase from Coryneform Bacterium Strain FG41: Mechanistic Implications for the Decarboxylase and Hydratase Activities. *Biochemistry*, 2013, **52**: 4830–4841.
2. **Youzhong Guo**, Hector Serrano, William H, Johnson, Jr., Steven Ernst, Marvin L. Hackert, Christian P. Whitman (2011) Crystal structures of native and inactivated cis-3-chloroacrylic acid dehalogenase: Implications for the catalytic and inactivation mechanisms. *Bioorg Chem.* **39**:1-9.

5. Biochemistry and molecular biology of termites: Formosan subterranean termites are the most aggressive and destructive timber pests in the United States. At Louisiana State University, working with Dr. Roger A. Laine and Dr. Gregg Henderson as a research assistant, my research focused on identification and cloning of novel proteins from the frontal gland of Fomosan termites. From separation of the secretions from the termite soldier to purification of proteins from the crude secretions and N-terminal sequencing and identification I successfully identified seven novel proteins from Formosan termites, including two lipocalins, two lysozymes, one proteinase inhibitor and two other function unknown proteins. My research work has led to two publications and may lead to discovery of novel methods in controlling termites.

1. Markus Hardt, **Youzhong Guo**, Gregg Henderson, Roger A. Laine. Zymogram with Remazol brilliant blue- labeled Micrococcus lysodeikticus cells for the detection of lysozymes: example of a new lysozyme activity in Formosan termite defense secretions. *Anal Biochem* **312**:73-6, 2003
2. Negulescu H, **Guo Y,** Garner TP, Goodwin OY, Henderson G, Laine RA, Macnaughtan MA A Kazal-Type Serine Protease Inhibitor from the Defense Gland Secretion of the Subterranean Termite Coptotermes formosanus Shiraki.*PloS One*. **10**: e0125376, 2015

Complete List of Published Work in **MyBibliography**:
https://www.ncbi.nlm.nih.gov/sites/myncbi/1fMGq6ZwVF6Q7/bibliography/53468019/public/?sort=date&direction=ascending.

D. Research Support

Ongoing Support

1. Project Number: 1R01GM132329-01 July 1st, 2019 - June 30th, 2023
Source: National Institutes of Health
Title: Native Cell Membrane Nanoparticles System

The major goals of this project are to develop a novel detergent-free system, the Native Cell Membrane Nanoparticles, for membrane protein research and demonstrate the capability of this system through high-resolution determination of selected model membrane proteins.
Role: PI

2. Membrane Protein Structural Biology
VCU startup
Role: PI

Pending Grant

1. Project Number: 1R01GM131068-01A1 (Resubmission based on prior 1R01GM131068-01)

Source: National Institutes of Health
Title: Interrogating native protein-lipid interaction of mechanosensitive channels using single-particle cryo-EM.
The major goals of this project are to determine high-resolution cryo-EM structures of selected representative mechanosensitive channels. The structural information of protein-lipid interaction obtained from this study will be combined to other biochemical and biophysical data to formulate proposal of the active mechanisms of mechanosensitive channels.
Role: Main collaborator
Project PI: Amedee des Georges, City College of New York