BIOGRAPHICAL SKETCH

NAME: Youzhong Guo

eRA COMMONS USER NAME: YZ2271

POSITION TITLE: Assistant Professor of Medicinal Chemistry

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Henan Normal University, Xinxiang, Henan, China	B.S.	07/1997	Biology
The University of Texas at Austin, Austin, TX	Ph.D.	05/2010	Medicinal Chemistry / Structural Enzymology
Columbia University, New York, NY	Postdoc	01/2016	Membrane Protein Structural Biology

A. Personal Statement

Research in my lab is engaged in the structural biology of membrane proteins. I am interested in deciphering the structures and functions of selected biologically or biomedically essential 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 the crystallization of membrane proteins. I have also developed an iodine phasing method for the structural determination of membrane proteins with X-ray crystallography. These methods have resulted in structural determinations of eight unique membrane proteins. I have also been developing a novel native cell membrane nanoparticle (NCMN) 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. For the first time, the well-organized high-resolution structure of lipid bilayer has been observed with single-particle cryo-EM. It is a revolutionary development for membrane protein structural biology and will likely profoundly affect structural biology and drug discovery and development.

With the recent advance of our NCMN system, we have developed a relatively large membrane-active polymer library. We can find suitable polymers to satisfy the specific requirement of unique membrane proteins. For example, we have recently used divalent ion compatible NCMN polymers determined high-resolution single-particle cryo-EM structure of human connexin 26 channel in the absence and presence of calcium ions at 2.6 2.9 Å, respectively. We have also found some suitable NCMN polymers for mechanosensitive channels through screening our NCMN polymer library. With proper NCMN polymers, we have solved preliminary high-resolution of MscS with entirely associated native lipids and all the transmembrane helices in a close state. We also found a proper NCMN polymer for YnaI, and our preliminary structure shows that with a small data-set, we can solve all the transmembrane-helices with some associated native lipids. I am pretty confident that we can reach our proposed goal to determine high-resolution cryo-EM structures of MscS and YnaI in their native lipid environment with complete protein-lipid interaction information with our advanced NCMN system.

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. Contributions 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. Furthermore, 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 the structure determination of GPCRs. A T4 lysozyme often replaces the third loop in GPCR. In contrast, single-particle cryo-EM has recently emerged as a compelling 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 been shown to be successful in the structural determination of a multiple drug transporter. We observed a high-resolution structure of the lipid bilayer itself, which we believe to be a first.

1. Kyle G. Kroeck, Weihua Qiu, Claudio Catalano, Thi Kim Hoang Trinh, **Youzhong Guo**. (2020) Native Cell Membrane Nanoparticles System for Membrane Protein-Protein Interaction Analysis. *J Vis Exp.* **161**, doi: 10.3791/61298. PubMed PMID: 32744521
2. 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 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. [^{11}C] PK11195 is a well-known Positron Emission Topography (PET) probe, specifically targeting TSPO. This technique has been used for diagnosing neuroinflammation and related diseases such as Alzheimer's disease. Also notable is that I discovered that TSPO is a novel enzyme in that it degrades protoporphyrin IX into bilindigin. This work resulted in a publication in *Science*. Furthermore, the new understanding of TSPO provides a factual 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.

2. 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 structure determination of membrane proteins. The lipid cubic phase (LCP) mimics a membrane protein bilayer, thus providing 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 have been crystallized in LCP. Another problem in determining novel crystal structures is phasing, and I also developed a novel and 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*. 8:15103. DOI: 10.1038/ncomms15103.

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), I worked with Dr. Christian P. Whitman and Dr. Marvin L. Hackert as a Ph. D student. My research focused on the tautomerase's structural enzymology superfamily. Enzymes within this superfamily are responsible for the degradation of organic chlorides. During that period, I successfully solved and deposited more than ten crystal structures for four distinct proteins into the Protein Data Bank, including two articles as the 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 primary 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*, **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 identifying and cloning novel proteins from the frontal gland of Formosan termites. I successfully identified seven novel proteins from Formosan termites. They include two lipocalins, two lysozymes, one proteinase inhibitor, and two other functionally unknown proteins. My research work has led to two publications and may lead to the 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. Additional Information: Research Support and/or Scholastic Performance

Ongoing Support

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

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

2. Project Number: 3R01GM132329-02S1 July 1st, 2020 - June 30th, 2021
Source: National Institutes of Health
Title: Proposal for an Administrative Supplement for Equipment Purchases for NIGMS Awardees NOT-GM-20-013 PA-18-591
The primary goal of this project is to purchase a GPC instrument for polymer characterization. NCMN Polymer development is part of the parent R01 grant 1R01GM132329

3. Membrane Protein Structural Biology
VCU startup
Role: P