BIOGRAPHICAL SKETCH

NAME: Youzhong Guo

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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

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 the structural determination of membrane proteins with X-ray crystallography. 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. This is the first time that the well-organized high-resolution structure of lipid bilayer has been observed with single-particle cryo-EM. I am interested in apply NCMN system to the investigation of the active mechanisms of mechanosensitive channels. MscS and MscL are the two model proteins that have been extensively investigated; however, the molecular mechanisms of gating are still not clear largely due to the lack of structural information of native protein-lipid interactions. Collaborating with Dr. Paul Blount (the PI of this proposal), we are interested in deciphering the molecular mechanisms of mechanosensitive channels. Taking advantage of our novel NCMN development, we have successfully determined the high-resolution single-particle cryo-EM structure of MscS with whole native lipids associated with the transmembrane domain. We can catch all of the natively associated cell membrane lipids with this system: central lipid plug, hook lipids and TM2-TM3 hairpin cavity lipids, and many other lipid molecules. We also developed a simple but robust NCMN-proteoliposome reconstitution system for the functional study of mechanosensitive channels, MscS and MscL. We demonstrated through three channels that this unique system may generally work for all membrane protein channels. We have recently successfully solved a high resolution cryo-EM structure of human connexin 26 with our NCMN system. I am confident that the proposed project can be accomplished with our improved quality of cryo-EM grid preparation.

B. Positions, Scientific Appointments, and Honors

Positions, Scientific Appointments

2016-Present: Assistant Professor: Institute for Structural Biology, Drug Discovery and Development, Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University

Honors

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

C. Contributions to Science

1. Native cell membrane nanoparticles system: 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, only a few such structures have been reported. 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 powerful method for determining membrane proteins and complexes' structure determination. 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. We observed a high-resolution structure of the lipid bilayer itself, which we believe to be a first. Besides, we also solved several other membrane protein structures together with native lipids, including *E.coli* mechanosensitive channels, MscS, YnaI, and human connexin channel 26. Furthermore, we also developed a unique NCMN-proteoliposome reconstitution system for membrane protein functional study.

1. Limin Yang, Claudio Catalano, Yunyao Xu, Weihua Qiu, Dongyu Zhang, Ann E. McDermott, Youzhong Guo, and Paul Blount. A native cell membrane nanoparticles system allows for high-quality functional proteoliposome reconstitution (2021) *BBA Advances* 1: 100011. https://doi.org/10.1016/j.bbadva.2021.100011.
2. 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
3. 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.
4. Claudio Catalano, Danya Ben-Hail, Weihua Qiu, Paul Blount, Amedee des Georges, **Youzhong Guo**. (2021). Cryo-EM Structure of Mechanosensitive Channel YnaI Using SMA2000: Challenges and Opportunities. Membranes, 11(11), 849. https://doi.org/10.3390/membranes11110849

2. Structure and activity of a 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 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.

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 the 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 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 crystallized in LCP. Another problem in determining novel crystal structures is phasing, and I also developed a novel and robust iodine phasing method for the crystallographic structure determination of membrane proteins.

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), 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 the 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 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 Fomosan termites. From the separation of the secretions from the termite soldier to the 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 discovering novel methods in controlling termites.

1. Markus Hardt, **Youzhong Guo**, Gregg Henderson, Roger A. Laine. (2003) 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**:73-76.
2. Horia Negulescu, **Youzhong Guo,** Thomas P. Garner, Octavia Y.Goodwin, Gregg Henderson, Roger A. Laine, Megan A. Macnaughtan.(2015) Kazal-Type Serine Protease Inhibitor from the Defense Gland Secretion of the Subterranean Termite *Coptotermes formosanus Shiraki.PloS One*. **10**: e0125376.

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

https://www.ncbi.nlm.nih.gov/myncbi/1fMGq6ZwVF6Q7/bibliography/public/