BIOGRAPHICAL SKETCH

NAME: Youzhong Guo

eRA COMMONS USER NAME: YZ2271

POSITION TITLE: Associate Professor of Medicinal Chemistry

EDUCATION/TRAINING

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

A. Personal Statement

My research focuses on membrane protein structural biology. I am interested in decoding the structures and functions of chosen physiologically or biomedically significant membrane proteins and complexes at the atomic level. While I use many current biophysical, biological, and computational techniques, single-particle cryo-EM and X-ray crystallography are my primary tools. I am also interested in developing novel approaches for the structural biology of membrane proteins. I created an efficient and cost-effective lipidic cubic phase crystallization method and an iodine phasing approach for determining the structure of membrane proteins using X-ray crystallography. I've also worked on a unique native cell membrane nanoparticle (NCMN) approach for single-particle cryo-EM high-resolution structural characterization. In 2017, I used this method to identify the structure of a multidrug transporter complexed with its native membrane lipid bilayer and observed that lipid molecules self-organize in the lipid bilayer in a hexagonal pattern. This is the first time a well-organized, high-resolution lipid bilayer structure has been detected using single-particle cryo-EM. We have successfully broadened the applicability of our NCMN technique to mammalian membrane proteins, and we have recently solved high-resolution single-particle cryo-EM structures of human connexin 26 gap junction channel and human transient receptor potential (TRP) channels, such as TRPML3. If funded, the proposed project will significantly upgrade the NCMN system to version 2.0, making it applicable to cholesterol-dependent mammalian membrane proteins, the prominent GPCR protein superfamily, and more dynamic membrane protein complexes. The current application is logically derived from my previous efforts. In summary, I have the expertise, leadership, training, expertise, and strong motivation necessary to carry out the proposed research project successfully.

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

R01 GM132329
Guo (PI)
07/01/2019-06/30/2024

Citations:

1. Trinh, T. K. H., Cabezas, A. J., Joshi, S., Catalano, C., Qiu, W., Deshmukh, S., des Georges, A., Guo, Y. (2023) pH-tunable membrane-active polymers, NCMNP2a-x, and their potential membrane protein applications. *Chemical Science* **14**, 7310–7326.

2. Catalano, C., Ben-Hail, D., Qiu, W., Blount, P., Georges, A.D., & Guo, Y. (2021) Cryo-EM Structure of Mechanosensitive Channel YnaI Using SMA2000: Challenges and Opportunities. *Membranes* **11**, 849.
3. Guo, Y. (2021) Detergent-free systems for structural studies of membrane proteins. *Biochem Soc Trans* **49**, 1361-1374.
4. Qiu, W., Fu, Z., Xu, G.G., Grassucci, R.A., Zhang, Y., Frank, J., Hendrickson, W.A., Guo, Y. (2018) Structure and Activity of Lipid Bilayer within a Membrane Protein Transporter. *Proc. Natl. Acad. Sci. USA* **115**, 12985-12990.

B. Positions, Scientific Appointments, and Honors

Positions, Scientific Appointments

2023-current: Associate Professor: Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University
2016-2023: Assistant Professor: 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 the 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 been shown to be successful in the structural determination of a multiple drug transporter. We observed a high-resolution lipid bilayer structure, which we believe to be a first. Besides, we also solved several other membrane protein structures 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. Trinh, T. K. H., Cabezas, A. J., Joshi, S., Catalano, C., Qiu, W., Deshmukh, S., des Georges, A., Guo, Y. (2023) pH-tunable membrane-active polymers, NCMNP2a-x, and their potential membrane protein applications. *Chemical Science* **14**, 7310–7326.
2. Guo, Y. (2021) Detergent-free systems for structural studies of membrane proteins. *Biochem Soc Trans*. **49**, 1361-1374.
3. Kroeck, K. G., Qiu, W., Catalano, C., Trinh, T.K.H., and Guo, Y. (2020) Native Cell Membrane Nanoparticles System for Membrane Protein-Protein Interaction Analysis. *J Vis Exp.* **161**, 10.3791/61298.
4. Qiu, W., Fu, Z., Xu, G.G., Grassucci, R.A., Zhang, Y., Frank, J., Hendrickson, W.A., Guo, Y. (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 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 [^{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 elucidates the molecular mechanism and biological role of TSPO in many physiological and pathological conditions.

1. Guo., Y., Kalathur, R. C., Liu, Q., Kloss, B., Bruni, R., Ginter, C., Kloppmann, E., Rost, B., and Hendrickson, W. A. (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. Su, M., Mao, Y., Yuan, Q., Gao, F., Li, D., Guo, Y. Yang, C., Wang, X., Bruni, R., Kloss, B., Zhao, H., Zeng, Y., Zhang, F., Marks, A., Hendrickson, W. A., Chen, Y. (2017) Structural basis for conductance through TRIC cation channels. *Nature Communication*. 8:15103. doi: 10.1038/ncomms15103.
2. Guo., Y., Kalathur, R. C., Liu, Q., Kloss, B., Bruni, R., Ginter, C., Kloppmann, E., Rost, B., and Hendrickson, W. A. (2015) Structure and activity of tryptophan-rich TSPO proteins. *Science* **347**: 551-554.
3. Yang, Y., Liu, Q., Kloss, B., Bruni, R., Ravi C. Kalathur, R. C., Guo, Y., Kloppmann, E., Rost, B., Henry M. Colecraft, H. M., Hendrickson, W. A. (2014) Structure and selectivity in bestrophin ion channels. *Science* **346**: 355-359.
4. Liu, Q., Guo, Y., Chang, Y., Cai, Z., Assur, Z., Mancia, F., Mark I. Greened, M.I., Wayne A. Hendrickson, W. A. (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. Guo, Y., Serrano, Poelarends, H. G., Johnson, W. H., Jr., Hackert, M. L., Whitman, C. P. (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. Guo, Y., Serrano, H., Johnson, W. H. Jr., Ernst, S., Hackert, M.L., Whitman, C. P., (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. Hardt, M., Guo, Y., Henderson, G., Laine, R. A. (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. Negulescu, H., Guo, Y., Garner, T. P., Goodwin, O. Y., Henderson, G., Laine, R. A., Megan A. Macnaughtan, M. A. (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/