BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Qiangjun Zhou

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

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
Central South University, Changsha, China	B.Eng.	2006	Pharmaceutical Engineering
University of Chinese Academy of Sciences, Beijing, China *(Formerly Graduate University of Chinese Academy of Sciences)*	Ph.D.	2012	Biochemistry and Molecular Biology
Stanford University, Stanford, California, USA	Postdoctoral Fellow	2019	Structural Biology and Neuroscience

A. Personal Statement

Scientific Interests

I am an assistant professor at Vanderbilt University, where I work in the Department of Cell and Developmental Biology, the Vanderbilt Brain Institute, and the Center for Structural Biology. My research program focuses on the molecular organization, dynamics, and functions of synaptic supramolecular assemblies at the nanoscale under near-native conditions. With a broad background in structural biology and molecular neuroscience, I have specific training and expertise in synaptic transmission and cellular structure biology, particularly in cryogenic electron tomography (cryo-ET). My graduate and postdoctoral research has resulted in numerous high-impact publications. As a postdoctoral fellow, I tackled a thirty-year-old challenge and successfully revealed the interactions and assembly of the pre-synaptic vesicle fusion machinery for neurotransmitter release. My work on multi-protein assemblies among the SNARE complex, a small regulatory factor complexin, and the calcium sensor Syt1 is considered a milestone in the field. I started my independent laboratory at Vanderbilt University in January 2020. Since arriving at Vanderbilt, I have made great strides in the face of unprecedented disruption caused by the COVID-19 pandemic, including lab shutdown, limited research capacity, as well as delay in shipping and installation of key equipment (Titan Krios TEM, FIB-SEM, and cryo-CLEM). After four years of delving into a new research direction (*in situ* cryo-ET) at Vanderbilt, I have established my own unique system and platform for *in situ* cryo-ET. Taking advantage of this technology and platform, my laboratory successfully discovered that postsynaptic proteins are organized into subsynaptic basic unit – we called PSD nanoblocks. These nanoblocks are heterogeneous in size, assembly and distribution, which likely underlies the dynamic nature of PSD to modulate synaptic strength. This finding provides a more comprehensive understanding of synaptic ultrastructure and suggests a potential mechanism for PSD nanoblocks to regulate synaptic strength. Additionally, we collaborated with the Skaar laboratory to reveal the molecular mechanism of membrane-bound ferrosome organelles containing non-crystalline iron phosphate biominerals in the human pathogen *Clostridioides difficile* using cryo-ET. We also made significant strides in the development of novel labeling techniques for 3D cryo-electron microscopy.

a. Pi H.*, Sun R.*, McBride J.R., Kruse A.R.S., Gibson-Corley K.N., Krystofiak E.S., Nicholson M.R., Spraggins J.M., **Zhou Q.[#],** Skaar E.P.[#], (2023) *Clostridioides difficile* ferrosome organelles combat nutritional immunity. **Nature** 623, 1009–1016. PMID: 37968387

b. Alten B., **Zhou Q.**, Shin O., Esquivies L., Lin P., White K.I, Sun R., Chung W.K., Monteggia L.M., Brunger A.T., Kavalali E.T. (2021) Role of aberrant spontaneous neurotransmission in SNAP25-associated encephalopathies. **Neuron** 109(1):59-72. PMCID: PMC7790958

c. **Zhou Q***, Zhou P*, Wang AL, Wu D, Zhao M, Südhof TC, Brunger AT (2017) The Primed SNARE-Complexin-Synaptotagmin Complex for Neuronal Exocytosis. **Nature** 548:420-425. PMCID: PMC5757840 (Featured on the cover)

d. **Zhou Q**, Lai Y, Bacaj T, Zhao M, Lyubimov AY, Uervirojnangkoorn M, Zeldin OB, Brewster AS, Sauter NK, Cohen AE, Soltis SM, Alonso-Mori R, Chollet M, Lemke HT, Pfuetzner RA, Choi UB, Weis WI, Diao J, Südhof TC, Brunger AT (2015) Architecture of the Synaptotagmin-SNARE Machinery for Neuronal Exocytosis. **Nature** 525:62-67. PMCID: PMC4607316

e. **Zhou Q***, Li J*, Yu H, Zhai Y, Gao Z, Liu Y, Pang X, Zhang L, Schulten K, Sun F and Chen C (2014), Molecular insights into the membrane-associated phosphatidylinositol 4-kinase IIα. **Nature Communications**, 5:3552. PMCID: PMC3974213
(* denotes co-first author, [#] denotes co-corresponding authors)

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2021-	Member, Vanderbilt Kennedy Center, Vanderbilt University, Nashville, TN
2020-	Core Faculty, Center for Structural Biology (CSB), Vanderbilt University, Nashville, TN
2020-	Training Faculty, Vanderbilt Brain Institute, Vanderbilt University, Nashville, TN
2020-	Assistant Prof., Dept. of Cell & Developmental Biology, Vanderbilt University, Nashville, TN
2017-2019	K99/R00 Research Associate, Stanford University, Stanford, CA
2013-2017	Postdoctoral Fellow, Howard Hughes Medical Institute, Stanford University, Stanford, CA

Honors

2021	"Neurodegenerative" TIPS initiative Award (P.I. Zhou & Alten), Vanderbilt University
2020	Faculty Fellowship Endowment Fund, Vanderbilt University
2019	Biophysical Journal Award for Outstanding Poster, Padova, Italy
2017	Pathway to Independence Award (K99/R00), National Institute of Mental Health
2016	Education Committee Travel Award, Biophysical Society 60th Annual Meeting
2012	Excellent Graduate Award, University of Chinese Academy of Sciences
2011	Outstanding Student Leader Award, University of Chinese Academy of Sciences
2010	Excellent Student Award, University of Chinese Academy of Sciences
2004-2006	University Scholarship, Central South University
2004-2006	China National Scholarship, China

C. Contributions to Science

1) Postsynaptic nanoblocks in excitatory synapses for trans-cellular alignment: Nanoscale organization of proteins within synapses is critical for maintaining and regulating synaptic transmission and plasticity. We use cryogenic electron tomography to directly visualize the *in situ* three-dimensional architecture and supramolecular organization of transsynaptic alignment of pre-cleft-postsynaptic components in their native cellular context in both synaptosomes from rat hippocampi and synapses from rat primary cultured neurons. High-resolution electron microscopy and quantitative analyses reveal that release sites align with adhesion molecules, receptor clusters, and postsynaptic density (PSD) nanoblocks as a physical, transsynaptic, nano-alignment. Additionally, it has been determined that the PSDs contain different-sized, membrane-associated, subsynaptic protein nanoblocks. Furthermore, large nanoblocks are formed by small nanoblocks positioned close enough together. Subtomogram averaging from synaptosomes showed

two types (type A and B) of postsynaptic receptor-like particles at resolutions of 24 Å and 26 Å, respectively. Our analysis suggested that potential presynaptic release sites are closer to nanoblocks with type A/B receptor-like particles than to nanoblocks without type A/B receptor-like particles, emphasizing the heterogeneity of nanoblocks in functional implications. The results of this study provide a more comprehensive understanding of synaptic ultrastructure and suggest that PSD is composed of clustering of various nanoblocks, and these nanoblocks are heterogeneous in size, assembly and distribution, which likely underlies the dynamic nature of PSD to modulate synaptic strength. One manuscript describing this study is under review. I am the corresponding author, and six trainees in my lab are coauthors (one postdoctoral fellow, one graduate student, two technicians, and two undergraduate students).

2) **Molecular mechanisms of ferrosome organelle formation in *Clostridioides difficile*:** Iron is indispensable for almost all forms of life but toxic at elevated levels. To survive within their hosts, bacterial pathogens have evolved iron uptake, storage, and detoxification strategies to maintain iron homeostasis. However, these iron homeostatic systems are largely undefined in the human pathogen *Clostridioides difficile*. *C. difficile* is a Gram-positive, spore-forming anaerobe and the leading cause of nosocomial and antibiotic-associated infections in the United States. In collaboration with Eric Skaar's laboratory at VUMC, we employed cryo-electron tomography to discover that *C. difficile* undergoes an intracellular iron biomineralization process and stores iron in membrane-bound ferrosome organelles containing non-crystalline iron phosphate biominerals. We further revealed that ferrosomes are often located adjacent to cellular membranes, as demonstrated through the visualization of lamellas prepared using focused ion beam scanning electron microscopy (FIB-SEM) followed by cryo-electron tomography analysis. I am the co-corresponding author and one postdoctoral trainee from my lab is a co-first author.

   a. Pi H.*, Sun R.*, McBride J.R., Kruse A.R.S., Gibson-Corley K.N., Krystofiak E.S., Nicholson M.R., Spraggins J.M., **Zhou Q.#,** Skaar E.P.#, (2023) *Clostridioides difficile* ferrosome organelles combat nutritional immunity. **Nature** 623, 1009–1016. PMID: 37968387

3) **Molecular mechanism of synaptic neurotransmitter release:** Ca^{2+}-triggered synaptic vesicle fusion is essential for neurotransmitter release. While it has been known for over twenty years that synaptotagmin (Syt), complexin (Cpx), and neuronal SNARE proteins play key roles in synchronous neurotransmitter release, how they cooperate to trigger synaptic vesicle fusion remained only partly understood. By designing novel constructs based on biochemical and biological relevant information, I determined the crystal structures of the SNARE–Syt1 complex and of the primed pre-fusion SNARE–Cpx–Syt1 complex**,** thereby revealing two essential interfaces that are required for synchronous release of neurotransmitters. In addition, I tested these interfaces by mutagenesis using electrophysiological recording techniques in cultured neurons. The complex structure explains the cooperativity between SNAREs, Cpx, and Syt1. Furthermore, combined with functional studies on the dominant-negative effects of certain Syt1 mutants, I proposed an unlocking mechanism that is triggered by Ca^{2+} binding to the Syt molecules, leading to SNARE complex zippering and membrane fusion. The crystal structures of the SNARE–Syt1 complex (*Nature* 2015) and of the primed pre-fusion SNARE–Cpx–Syt1 complex (*Nature* 2017) are considered as a milestone over a long history of structural studies in this field and it sets the framework for a better understanding of the system. I was the first or co-first author in both publications in 2015 and 2017. In addition, I trained one of my co-author (a research technician) and supervised his work on the project.

   Based on a fragment of SNAP-25 that participates in the interface as observed in the crystal structure of the SNARE-Syt1 complex, we designed a hydrocarbon-stapled peptide to specifically inhibit Ca^{2+}-triggered vesicle fusion with reconstituted neuronal SNAREs and Syt1, as well as Ca^{2+}-triggered vesicle fusion with reconstituted airway SNAREs and Syt2 for the airway system. This engineered stapled peptide enters airway epithelial cells to inhibit fusion of the secretory granule with the cell membrane and blocking mucin secretion in IL-13-primed airway epithelial cells *in vitro* and *in vivo* in mice (*Nature* 2022). It paved the way for the development of therapeutics for mucus-associated lung disease. This study also further demonstrated that the primary interface is the universal binding mode for fast Ca^{2+} sensors (Sty1, Syt2, Syt9) and SNARE complexes. The engineered peptide was designed based on the crystal structures that I determined. And I also made contributions to design the engineered peptides.

a. Inhibition of calcium-triggered secretion by hydrocarbon-stapled peptides. Lai Y, Fois G, Flores JR, Tuvim MJ, **Zhou Q**, Yang K, Leitz J, Peters J, Zhang Y, Pfuetzner RA, Esquivies L, Jones P, Frick M, Dickey BF, Brunger AT (2022) **Nature** 603(7903):949-956. PMID: 35322233

b. **Zhou Q***, Zhou P*, Wang AL, Wu D, Zhao M, Südhof TC, Brunger AT (2017) The Primed SNARE-Complexin-Synaptotagmin Complex for Neuronal Exocytosis. **Nature** 548:420-425. PMCID: PMC5757840 (Featured on the cover)

c. **Zhou Q**, Lai Y, Bacaj T, Zhao M, Lyubimov AY, Uervirojnangkoorn M, Zeldin OB, Brewster AS, Sauter NK, Cohen AE, Soltis SM, Alonso-Mori R, Chollet M, Lemke HT, Pfuetzner RA, Choi UB, Weis WI, Diao J, Südhof TC, Brunger AT (2015) Architecture of the Synaptotagmin-SNARE Machinery for Neuronal Exocytosis. **Nature** 525:62-67. PMCID: PMC4607316
(* denotes co-first author)

4) **Aberrant spontaneous release of neurotransmitters causes developmental and epileptic encephalopathies (DEEs) of infancy and childhood:** Synaptic dysfunction in neurological disorders is the cumulative outcome of physiological synaptic structure and function disturbances, and the unexpected commonality of molecular and cellular mechanisms operating under neurodevelopmental and neurodegenerative conditions, most of which meet at the synaptic level. Spontaneous release is a major mode of synaptic transmission, one that is independent of presynaptic action potentials. However, the physiological roles of spontaneous release in neurodevelopmental diseases are currently uncertain, presenting challenges to the understanding and treatment of neurological disorders. With the advent of next-generation sequencing, ten different heterozygous, mostly *de novo*, mutations have been identified in *SNAP25* in patients with clinically heterogeneous DEEs. SNAP25 is a component of the SNARE complex, which accounts for the specificity of membrane fusion for neurotransmitter release, including spontaneous release. As part of a collaborative project, we identified the molecular mechanisms of SNAP25-associated DEEs by using electrophysiological, structural, and biochemical approaches. Our collaborative effort showed that apart from impaired evoked release, aberrant spontaneous release, the non-canonical action potential-independent form of neurotransmitter release, is responsible for disease heterogeneity. Furthermore, we identified a single disease-associated variant that increases spontaneous release without affecting an evoked release. To our knowledge, this was the first evidence implicating spontaneous release as a cause of any disease of humans so far. I contributed to the design of the study, supervised, and executed the biochemical experiments and structure determination, as well as some of the electrophysiology experiments.

a. Alten B., **Zhou Q.**, Shin O., Esquivies L., Lin P., White K.I, Sun R., Chung W.K., Monteggia L.M., Brunger A.T., Kavalali E.T. (2021) Role of aberrant spontaneous neurotransmission in SNAP25-associated encephalopathies. **Neuron** 109(1):59-72. PMCID: PMC7790958

5) **Multi-protein and protein-membrane assemblies:** My graduate work focused primarily on high resolution structure determination of protein complexes and membrane-associated proteins to reveal multi-protein and protein-membrane assemblies. My thesis work focused on investigating the structural basis of phosphatidylinositol 4-kinase IIα (PI4KIIα), which plays a central role in cell signaling and membrane trafficking. Critical to PI4KIIα's function is its association with membranes. Structural and functional studies of PI4KIIα revealed the structural basis for protein-membrane interactions in addition to revealing its activity is regulated indirectly through changes in the membrane environment (***Nature Communications*** 2014).

a. **Zhou Q**, Li J, Yu H, Zhai Y, Gao Z, Liu Y, Pang X, Zhang L, Schulten K, Sun F and Chen C (2014) Molecular insights into the membrane-associated phosphatidylinositol 4-kinase IIα. **Nature Communications** 5:3552. PubMed PMID: 24675427; PubMed PMCID: PMC3974213.

b. **Zhou Q**, Zhai Y, Lou J, Liu M, Pang X, Sun F (2011) Thiabendazole inhibits ubiquinone reduction activity of mitochondrial respiratory complex II via a water molecule mediated binding feature. **Protein & Cell** (7):531-542. PubMed PMID: 21822798.

Complete List of Published Work:
https://www.ncbi.nlm.nih.gov/myncbi/qiangjun.zhou.1/bibliography/public/