BIOGRAPHICAL SKETCH

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NAME: Xuewu Zhang

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

POSITION TITLE: Professor, Department of Pharmacology, UT Southwestern Medical Center

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
Wuhan University, China	B.S.	07/1995	Biology
Shanghai Institute of Plant Physiology, China	M.S.	06/1998	Plant Physiology
Albert Einstein College of Medicine, New York	Ph.D.	04/2003	Immunology, Structural Biology
Department of Molecular and Cell Biology, University of California, Berkeley		09/2007	Structural biology, Signal transduction

A. Personal Statement

My scientific career has been mostly devoted into studying transmembrane receptors. As a graduate student, I determined the structures of the extracellular regions of several immune co-stimulatory/inhibitory receptors and ligands and their complexes, including CTLA4 and PD1. These structures served as the basis for the development of the currently most promising cancer immunotherapies. My postdoctoral work revealed that the intracellular kinase domain of the epidermal growth factor receptor (EGFR) forms an asymmetric dimer, in which one kinase domain acts as the allosteric activator for the other. This new paradigm underlies the activation of the EGFR family members through homo- and hetero-dimerization. Similar mechanisms are now found to drive the activation of many other kinases. My own independent laboratory has been studying plexins, the cell surface receptors for the guidance molecules semaphorins. Much of the current mechanistic understanding of plexin has emerged from my laboratory. We identified the Rap GTPase as the substrate for the plexin intracellular GTPase activating protein (GAP) domain, which is the central component of plexin signaling. We were the first to report the crystal structures of an intact plexin intracellular region, as well as many structural and mechanistic analyses of the associated signaling complexes. More recently, we have employed cryo-electron microscopy (cryo-EM) to study the large assemblies of semaphorin in complex with the extracellular region and full-length plexin.

Another major focus of my lab is directed at innate immunity, in particular the cGAS-STING pathway (1-3). STING is an ER membrane protein that plays an essential role in this pathway. Recent studies have shown that activation of STING is a promising approach for boosting the immune system for cancer therapy. STIN is activated by cyclic di-nucleotide such as cyclin GMP-AMP (cGAMP) and transduces signal to activate the expression of type I interferon and other innate immune responses. In collaboration with Drs. Xiaochen Bai (Co-PI of this multiple-PI application) and Zhijian James Chen at UTSW, we have reported in 2019 the first set of cryo-EM structures of full-length STING and its complex with the downstream kinase TBK1. These studies revealed how STING is activated by cGAMP and how it triggers TBK1 activation. During these studies, we have accumulated expertise for mechanistic analyses of Sting with both the structural and functional approaches. In 2022, the Zhang and Bai labs together reported the cryo-EM structure of human STING bound to both cGAMP and C53, which targets a novel pocket in the transmembrane region of STING. The proposed project is based on this exciting new finding and other progress that we have made recently, aimed at deeper understanding of the regulatory mechanisms and provide lead compounds targeting STING for cancer therapy.

1. Shang G*, Zhang C*, Chen ZJ#, Bai XC# and **Zhang X#** (2019). Cryo-EM structures of STING reveal its mechanism of activation by cyclic GMP-AMP. Nature *567*, 389-393. PMCID: PMC6859894

2. Zhang C*, Shang G*, Gui X, **Zhang X#**, Bai XC# and Chen ZJ# (2019). Structural basis of STING binding with and phosphorylation by TBK1. Nature *567*, 394-398. PMCID: PMC6862768

3. Lu D*, Shang G*, Li J, Lu Y, Bai XC# and **Zhang X#** (2022). Activation of STING by targeting a pocket in the transmembrane domain. Nature 604, 557-562. PMID: 35388221.

B. Positions, Scientific Appointments, and Honors

2002	Julius Marmur Research Award, Albert Einstein College of Medicine
2003-2007	Postdoctoral Associate, John Kuriyan's laboratory, UC Berkeley
2007-2014	Assistant Professor, UT Southwestern Medical Center
2007	Virginia Murchison Linthicum Scholar, UT Southwestern Medical Center
2014-2020	Associate Professor with tenure, UT Southwestern Medical Center
2019-2023	Maximizing Investigator's Research Award (MIRA) (R35), NIGMS
2019-	Editorial board, Scientific Reports
2020-	Full professor, UT Southwestern Medical Center
2009-	Member of Cell signaling and trafficking structures section, Faculty1000
2011-	Ad Hoc reviewer for Argonne National Laboratory General User Proposal
2013	Grant review, Israel science foundation
2013, 2015	Grant review, Austria Science Fund
2014	Grant review, MRC (UK)
2014	Grant review, Ad Hoc, NIH Macromolecular Structure and Function study section C
2014	Session chair, Gordon research seminar, Phosphorylation and G-protein-mediated signaling
2015	Grant review, NIH ZRG1 F04B-D(20) Biophysics Fellowship study section
2016-2019	Grant review, proteins and crystallography committee, American Heart Association
2018-	Grant review, Research Grants Council (RGC), Hong Kong
2018	Grant review, Ad Hoc, NIH Macromolecular Structure and Function study section A
2019	Grant review, ZRG CB-B(55) special emphasis panel, NIGMS, NIH
2020	Grant review, Macromolecular structure and function study section A, NIH
2020	Grant review, MIRA special emphasis panel, ZRG1 CB-V(55) R, NIGMS, NIH
2021-	Standing member, NIGMS MRAB study section, NIH

C. Contributions to Science

(*equal contributions; #co-corresponding authors).

C1. Signaling mechanisms of co-stimulator/inhibitory receptors in T cells. The immune response of T cells is initiated by the recognition by the T Cell Receptor (TCR) of an antigenic peptide in complex with the major histocompatibility complex (MHC). Full activation of T cells requires a secondary signal, delivered by the so-called co-stimulatory receptors such as CD28 upon binding to their ligands such as B7. Another class of receptors related to CD28, including CTLA-4 and PD-1, negatively regulates T cell signaling. This negative regulation is essential for turning off immune response after pathogen elimination as well as preventing autoimmunity. As a graduate student, I determined the crystal structures of CTLA-4, its ligand B7-2 and the CTLA-4/B7-2 complex (Schwartz and Zhang et al, 2001, Nature; Zhang and Schwartz et al, 2003, PNAS). These structures together revealed the atomic detail of how B7-2 binds CTLA-4, and more importantly, suggested a mechanism of CTLA-4 activation by formation of a large, alternating array of CTLA-4 and B7 dimers on the cell surface. I also solved the first crystal structure of PD-1, and determined its oligomerization state and mapped its ligand-binding site (Zhang et al, 2004, Immunity). These studies established the framework for understanding the signaling mechanisms of the co-stimulatory/inhibitory receptors. Anti-CTLA-4 and anti-PD1 antibodies have

recently shown great promise in treating cancer. My work on CTLA-4 and PD-1 has served as the structural basis for some of these developments.

Schwartz JC*, **Zhang X***, Fedorov AA, Nathenson SG, Almo SC. (2001). Structural basis for co-stimulation by the human CTLA-4/B7-2 complex. **Nature,** 410, 604-608.

Zhang X*, Schwartz JC*, Almo SC, Nathenson SG. (2003). Crystal structure of the receptor-binding domain of human B7-2: insights into organization and signaling. **Proc Natl Acad Sci U S A,** 100, 2586-2591.

Zhang X, Schwartz JC, Guo X, Bhatia S, Cao E, Lorenz M, Cammer M, Chen L, Zhang ZY, Edidin MA, Nathenson SG, Almo SC. (2004). Structural and functional analysis of the costimulatory receptor programmed death-1. **Immunity,** 20, 337-347.

C2. Regulatory mechanisms of the epidermal growth factor receptor (EGFR). EGFR is the first identified receptor tyrosine kinase and has been the model system for studying receptor-mediated signaling. Dysregulation of EGFR and its homologues (ErbB2, ErbB3 and ErbB4) is causatively linked to many types of cancer such as lung cancer and glioblastoma. Several antibodies and small molecule inhibitors are clinically used for treating these diseases. While it was known that EGFR is activated by EGF-induced dimerization, the underlying mechanism by which the dimerization drives activation of the kinase had remained elusive. My postdoctoral work revealed that the kinase domain of EGFR forms an asymmetric dimer, in which one kinase domain acts as the allosteric activator for the other (Zhang et al., 2006, Cell). This new paradigm underlies the activation of the EGFR family members through homo- and hetero-dimerization. Particularly, it explains how ErbB3, an intrinsically dead kinase, acts as a potent activator for other family members through hetero-dimerization and in some cases causes cancer. This activation mechanism also rationalizes the effects of many cancer-driving mutations of the EGFR family members found in patients. I also determined the crystal structure of the EGFR kinase domain in complex with Mig6, a negative-feedback inhibitory protein of EGFR (Zhang et al, 2007, Nature). The structure and associated biochemical analyses revealed that one of the mechanisms by which Mig6 inhibits EGFR is to block the formation of the asymmetric dimer. This work suggests a new strategy for designing highly specific inhibitors of EGFR.

Zhang X, Gureasko J, Shen K, Cole PA, Kuriyan J. (2006). An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. **Cell,** 125, 1137-1149.

Zhang X, Pickin KP, Bose R, Jura N, Cole PA, Kuriyan J. (2007). Inhibition of the EGF receptor by binding of MIG6 to an activating kinase domain interface. **Nature,** 450, 741-744.

Jura N, Endres NF, Engel K, Deindl S, Das R, Lamers MH, Wemmer DE, **Zhang X**, Kuriyan J. (2009). Mechanism for activation of the EGF receptor catalytic domain by the juxtamembrane segment. **Cell,** 137, 1293-307. PMCID: PMC2814540

Shang Y, Eastwood MP, **Zhang X**, Kim ET, Arkhipov A, Dror RO, Jumper J, Kuriyan J, Shaw DE (2012). Oncogenic Mutations Counteract Intrinsic Disorder in the EGFR Kinase and Promote Receptor Dimerization. **Cell,** 149(4):860-70. PMID: 22579287

C3. Structures and Signaling mechanisms of plexin. My own laboratory has been focused on plexin, the primary cell surface receptor for the guidance molecules semaphorin. Plexin signaling plays essential roles in regulating processes such as the development of the nervous and cardiovascular systems. Malfunction of plexin has been associated with neurological disorders and cancer. By the time my laboratory entered this field, little was known about the molecular mechanisms by which plexin is regulated and transduces signal. We determined the first structure of the full-length intracellular region of a plexin family member (He et al, 2009, PNAS). A previous paradigm was that plexin transduces signal by acting as a GTPase activating protein (GAP) for the Ras homolog R-Ras. We shifted this paradigm by showing that plexin is actually active to Rap, not R-Ras (Wang et al, 2012, Science Signaling). The RapGAP activity was unanticipated, because plexin is not related to canonical RapGAPs and does not have the associated catalytic machinery. We determined the structures of the active dimer of PlexinC1, both in the apo-state and in complex with Rap (Wang et al, 2013, eLife). These structures elucidated how dimerization activates plexin, and how plexin catalyzes GTP hydrolysis for Rap though a non-canonical mechanism. The structures explain the unique specificity between plexin and Rap. Our functional analyses confirmed that the RapGAP activity of plexin is essential for the axon guidance function (Wang et al, 2012, Science Signaling). More recently, we reported the first high-resolution Cryo-EM structure of a full-length plexin in complex with its ligand, providing unprecedented near complete view of the ligand-induced dimerization of plexin (Kuo et al, 2020, Nature Comm.).

He H, Yang T, Terman JR, **Zhang X** (2009). Crystal structure of the plexin A3 intracellular region reveals an autoinhibited conformation through active site sequestration. **Proc Natl Acad Sci U S A,**106, 15610-15615. PMCID: PMC2747167

Wang Y*, He H*, Srivastava N, Vikarunnessa S, Chen Y, Jiang J, Cowan CW, **Zhang X.** (2012). Plexins are GTPase Activating Proteins for Rap and Activated by Induced Dimerization. **Science Signaling,** 5, ra6. PMCID: PMC3413289

Wang Y*, Heath PG*, Brautigam CD, He H, **Zhang X.** (2013). Structural Basis for activation and non-canonical catalysis of the Rap GTPase activation protein domain of plexin. **eLife** 2, e01279. PMCID: PMC3787391

Kuo, Y.C.*, Chen, H.*, Shang, G.*, Uchikawa, E., Tian, H., Bai, X.C.#, and Zhang, X#. (2020). Cryo-EM structure of the PlexinC1/A39R complex reveals inter-domain interactions critical for ligand-induced activation. **Nature communications** 11, 1953. PMCID: PMC6859894.

C4. Interactions of plexins with regulatory proteins. Some plexins require the Neuropilin (Nrp1) co-receptor for high affinity binding to semaphorin. We published the first cryo-EM structure of a near intact 2:2:2 semaphorin/plexin/neuropilin extracellular assembly (Lu et al, 2021, Nature Commun.), revealing how each protein in the complex makes multiple interfaces with others to stabilize the large assembly to drive the dimerization and activation of plexin. Plexin signaling is also regulated by many intracellular proteins. Using both X-ray crystallography and MD simulations, we published a model to explain how two similar Rho-family GTPase exert opposite regulatory effects on plexin (Liu et al, 2021, eLife). We have also structurally characterized the interaction between plexins with two families of PDZ-domain containing proteins, PDZ-RhoGEF and GIPC, which contribute to plexin signaling and endocytosis respectively (Pascoe et al, 2015, PNAS; Shang et al, 2017, eLife). These structures not only elucidated how PDZ-RhoGEF and GIPC bind plexin, but suggested a general mechanism for enhancing specificity in PDZ-mediated interactions. The GIPC proteins are universal adaptor proteins that tether cargo proteins such as PlexinD1 to the myosin VI motor for endocytic trafficking. We discovered how GIPCs are autoinhibited, and how they are activated by cargo binding (Shang et al, 2017, eLife). Myosin VI is known to oligomerize for enhanced mechanical force and processive transport. We found that GIPCs and myosin VI form an alternating linear array mediated by mutual bi-valent interactions, which underlies their oligomerization in cells (Shang et al, 2017, eLife).

Lu D*, Shang G*, He X*, Bai XC# & **Zhang X#** (2021). Architecture of the Sema3A/PlexinA4/Neuropilin tripartite complex. **Nature communications** 12, 3172, doi:10.1038/s41467-021-23541-x. PMCID: PMC8155012

Liu Y, Ke P, Kuo YC, Wang Y, **Zhang X#**, Song C# & Shan Y# (2021). A putative structural mechanism underlying the antithetic effect of homologous RND1 and RhoD GTPases in mammalian plexin regulation. **Elife** 10, doi:10.7554/eLife.64304. PMCID: PMC8219378.

Pascoe HG, Gutowski S, Chen H, Brautigam CA, Chen Z., Sternweis PC and **Zhang X** (2015). A secondary PDZ domain-binding site on class B plexins enhances the selectivity towards PDZ-RhoGEF. **Proc Natl Acad Sci U S A**, 112(48):14852-7. PMCID: PMC4672773

Shang G., Brautigam CA, Chen R, Lu D, Torres-Vázquez J and **Zhang X** (2017). Structural analyses reveal a regulated oligomerization mechanism of the PlexinD1/GIPC/myosin VI complex. **eLife**, 10.7554/eLife.27322. PMCID: PMC5461112

C5. Regulatory mechanism of the innate immunity adaptor STING. Cells, including non-immune cells, can sense DNA viruses or bacteria and launch immune responses to them by detecting pathogen DNA in the cytosol. The molecule basis of this ubiquitous signaling pathway has been elucidated recently. The cytosolic DNA sensor cyclic-GMP-AMP (cGAMP) synthase (cGAS) is activated by cytosolic DNA and synthesizes the second messenger cGAMP. cGAMP activates the adaptor protein STING, which binds and activates the TBK1 kinase. TBK1 phosphorylates both STING and the transcription factor IRF3, which promotes expression of type I interferons. In collaboration with the labs of Drs. Zhijian (James) Chen and Xiao-chen Bai, we used cryo-EM to determine the first set of structures of full-length STING, as well as its complex with TBK1 (Shang et al, 2019, Nature; Zhang et al, 2019, Nature). These structures together clearly elucidate the mechanisms by which cGAMP activates STING and triggers its recruitment and activation of TBK1 (Zhang et al, 2021, Immunity). More recently, in collaboration with Dr. Bai, we discovered a cryptic ligand binding site in the transmembrane region of STING (Lu et al, 2022, Nature). We found that a small molecule compound binding to this new site can activate STING independently, as well as synergistically with cGAMP. These results pave the way for the development of novel STING agonists and antagonists for therapeutic purposes.

Shang G*, Zhang C*, Chen ZJ#, Bai X#, and **Zhang X#** (2019). Cryo-EM structures of full-length STING reveal its mechanism of activation by cyclic GMP-AMP. **Nature**, 567(7748): 389-393. Doi: 10.1038/s41586-019-0998-5. PMCID: PMC6859894

Zhang C*, Shang G*, **Zhang X#**, Bai X# and Chen ZJ# (2019). Structural basis of STING binding with and phosphorylation by TBK1. **Nature**, 567(7748): 394-398. Doi: 10.1038/s41586-019-1000-2. PMCID: PMC6862768

Zhang X#, Bai X# and Chen ZJ# (2020). Structure and mechanisms in the cGAS-STING innate immunity pathway. **Immunity** 53, 43-53. PMID: 32668227

Lu D*, Shang G*, Li J, Lu Y, Bai X# and **Zhang X#** (2022). Activation of STING by targeting a pocket in the transmembrane domain. **Nature**, doi:10.1038/s41586-022-04559-7. PMID: 35388221

A full list of my published work can be found at:

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

BIOGRAPHICAL SKETCH

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NAME: Bai, Xiao-chen

eRA COMMONS USER NAME (credential, e.g., agency login): s167380

POSITION TITLE: Associate 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
Tsinghua University, Beijing, China	BME	07/2004	Mechanical engineering
Tsinghua University, Beijing, China	PHD	07/2011	Biophysics
MRC, Laboratory of Molecular Biology, Cambridge, UK	Postdoctoral Fellow	02/2017	Single particle cryo-EM

A. Personal Statement

I have been working on cryo-EM method development and structural determination for more than a decade. I was involved in establishing the first cryo-EM facility in China, when I was a Ph.D. student in Tsinghua University. While I was a postdoc in Sjors Scheres lab at MRC-LMB in UK, I invented entirely new techniques that dramatically improved the capabilities of cryo-EM technology. This includes the optimization of the direct electron detector “Falcon” and devising a new algorithm to correct for beam-induced particles motion. I also devised a modified classification algorithm —focused classification with density subtraction, which can be used to identify local conformational changes within a large complex. My effort during my postdoc resulted in various high-resolution cryo-EM structures from large protein complexes, such as ribosomes, to small membrane proteins, such as ion channel and γ-secretase. After starting my independent lab at UTSW in 2017, I continued working on the structural and functional studies of ion channels and have solved the cryo-EM structures of several important ion channels and transporters — TRPML1, TRPM4, TPC1/2, MCU, KCC1 and ZnT8. Recently, I was able to solve the structures of full-length STING, a membrane protein that is essential in innate immune response, in apo, active and agonist bound states. In the meantime, I have established a highly productive research program on the structural and functional studies of receptor tyrosine kinases (RTKs), the essential proteins for diverse physiological processes.

B. Positions, Scientific Appointments, and Honors

Positions

2021 - Associate Professor, UT Southwestern Medical Center, Dallas, TX
2017 - 2021 Assistant Professor, UT Southwestern Medical Center, Dallas, TX

Honors

2022 SCBA Kenneth Fong Young Investigator Award
2021 Amgen Young Investigators Award
2017 Virginia Murchison Linthicum Scholar, UT Southwestern Medical Center
2017 CPRIT Scholar, Cancer Prevention and Research Institute of Texas
2015 MRC CEO Award, MRC, UK
2013 - 2014 Marie Curie Fellow, European Commission

C. Contributions to Science

1. **Developing new methods for cryo-EM image processing**: I have been involved in a number of projects on cryo-EM method development when I was a postdoc in Sjors Scheres lab at MRC-LMB. To tackle the problem that the electron beam induced protein particles motion will lead to image blurring, I was the first person to collect and process full data sets with the movie capabilities of the new direct electron detector "Falcon", showing that realignment of individual movie frames could correct for particle movements and thereby improve the signal:noise ratios of individual protein particle images. This has since turned out to be a real game-changer for cryo-EM structure determination. The results of this work showed for the first time that movie-processing allowed near-atomic resolution cryo-EM reconstructions from small cryo-EM dataset. I also worked together with my postdoc supervisor Sjors Scheres to establish an entire workflow for using RELION to process single particle cryo-EM data. In addition, through developing a modified classification algorithm —focused classification with density subtraction, I showed that the conformations of local flexible regions in a large protein complex can be classified into different states, which allows for an unprecedented view of protein dynamics.

    a. **Bai XC**, Rajendra E, Yang G, Shi Y, Scheres SH. Sampling the conformational space of the catalytic subunit of human γ-secretase. **Elife**. 2015 Dec 1; PMCID: PMC4718806
    b. **Bai XC**, Fernandez IS, McMullan G, Scheres SH. Ribosome structures to near-atomic resolution from thirty thousand cryo-EM particles. **Elife**. 2013 Feb 19;2:e00461. PMCID: PMC3576727
    c. **Bai XC**, Martin TG, Scheres SH, Dietz H. Cryo-EM structure of a 3D DNA-origami object. **Proc Natl Acad Sci U S A**. 2012 Dec 4;109(49):20012-7. PMCID: PMC3523823
    d. **Bai XC**†, Yan C, Yang G, Lu P, Ma D, Sun L, Zhou R, Scheres SHW†, Shi Y†. An atomic structure of human γ-secretase. **Nature**. 2015 Sep 10;525(7568):212-217. PMCID: PMC4568306 **(†Correspondence author)**

2. **Using cryo-EM to determine the structures of macromolecular machines**: I have determined a variety of high-resolution cryo-EM structures of large protein complexes, such as ribosomes, apoptosomes, human cohesin/DNA complex and STRIPAK complex. I solved the cryo-EM structure of yeast mitochondrial ribosome large subunit at a resolution of 3.2 Å. This was the first time that the atomic structure of a large asymmetric complex was obtained by using cryo-EM, and thus was marked as the beginning of "resolution revolution" in cryo-EM field. Through my cryo-EM analysis of Plasmodium falciparum ribosomes bound with drugs, where and how two different types of antimalarial drugs (Emetine and Mefloquine) bind in this parasitic ribosome were revealed. Recently, I solved the high-resolution structure of cohesin/NIPBL/DNA complex, providing a blueprint to understand how NIBPL and DNA synergistically activate cohesin, which represents a milestone in cell division field.

    a. Amunts A*, Brown B*, **Bai XC***, Llácer JL*, Hussain T, Emsley P, Long F, Murshudov G, Scheres SHW, Ramakrishnan V. Structure of the Yeast Mitochondrial Large Ribosomal Subunit. Science. 2014. Vol. 343, Issue 6178. PMCID: PMC4046073. (***These authors contributed equally to the work.)**
    b. Wong W*, **Bai XC***, Brown A, Fernandez IS, Hanssen E, Condron M, Tan YH, Baum J, Scheres SH. Cryo-EM structure of the Plasmodium falciparum 80S ribosome bound to the anti-protozoan drug emetine. **Elife**. 2014 Jun 9;3 PMCID: PMC4086275 (***These authors contributed equally to the work.)**
    c. Shi ZB, Gao HS, **Bai XC†**, Yu HT†. Cryo-EM structure of the human cohesin-NIPBL-DNA complex. **Science**. 2020. PMID: 32409525 **(†Correspondence author)**
    d. Jeong BC, Bae SJ, Ni L, Zhang XW, **Bai XC†**, Luo X**†**. Cryo-EM structure of the Hippo signaling integrator human STRIPAK. **Nat Struct Mol Biol**. 2021. Vol. 28, Issue 3. PMCID: PMC8315899 **(†Correspondence author)**

3. **Structural study of membrane proteins that are critical for signaling**: The cGAS-cGAMP-STING pathway is a universal mechanism for different type of cells to fight microbial infection. Through the collaboration with James Chen and Xuewu Zhang labs, we determined the high-resolution structures of full-length STING with and without cGAMP bound, as well as in the higher order oligomeric state. These STING structures in different states provide some intriguing clues about how the conformational change of STING upon cGAMP binding trigger STING oligomerization and activation. We also determined the structure of STING in complex with TBK1, a kinase which can phosphorylate STING to trigger the downstream signaling. This structural information, together with biochemical and cellular based analysis,

leads us to understand why the recruitment of multiple TBK1 dimers by STING higher order oligomers is critical for STING activation. Very recently, we determined the cryo-EM structure of STING bound with both cGAMP and an agonist – C53, revealing a novel agonist site in the TMD of STING. I also determined the cryo-EM structures of Scap, a cholesterol-sensing protein, alone or in complex with Insig. These structures reveal that the binding of Scap to Insig triggers a large rotation of the luminal L1-L7 domain of Scap, hinting how cholesterol blocks Scap's transport of SREBPs.

a. Shang G, Zhang C, Chen ZJ†, **Bai XC†**, Zhang XW†. Cryo-EM structures of STING reveal its mechanism of activation by cyclic GMP-AMP. **Nature**. 2019 Mar;567(7748):389-393. PMCID**:** PMC6859894 **(†Correspondence author)**

b. Zhang C, Shang G, Gui X, Zhang X†, **Bai XC†**, Chen ZJ†. Structural basis of STING binding with and phosphorylation by TBK1. **Nature**. 2019 Mar;567(7748):394-398. PMCID: PMC6862768 **(†Correspondence author)**

c. Kober DL, Radhakrishnan A†, Goldstein JL, Brown MS, Clark LD, **Bai XC†**, Rosenbaum DM†. Scap structures highlight key role for rotation of intertwined luminal loops in cholesterol sensing. **Cell**. 2021. Vol 184. Issue 14. PMCID: PMC8277531 (**†Correspondence author**)

d. Lu D, Shang G, Li J, Lu Y, **Bai XC†**, Zhang XW†. Activation of STING by targeting a pocket in the transmembrane domain. **Nature**. 2022 Apr; 604(7906):557-562. PMID: 35388221. (**†Correspondence author**)

4. **Structural and functional studies of ion channels/transporters**: Ryanodine receptors (RYRs), a class of calcium channels that release calcium from cellular organelles, is the first ion channel I chose to work on, when I was a postdoc in MRC-LMB. By using different buffer conditions, I managed to determine the high-resolution cryo-EM structures of RYR in both close and open states. After moving to UTSW, I continued working on other ion channels/transports through close collaboration with Youxing Jiang lab. I managed to solve the structures of several important ion channels, including TRPML1, TRPM4, TPC1/2 and MCU complex, as well as transporters, such KCC1 and ZnT8. As one example, TPC1 is a lysosomal channel that plays critical roles in vesicular fusion and trafficking, and is activated by $PI(3,5)P_2$. I determined the cryo-EM structures of mouse TPC1 in both of apo and $PI(3,5)P_2$ bound states, unveiling a large conformational change of pore-forming helix upon $PI(3,5)P_2$ binding. Recently, I determined the structures of human ZnT8, a zinc transporter that is located at insulin secretory granules and linked to diabetes, in both outward- and inward-facing conformations. This work provides the structural insights into the Zn^{2+}/H^{+} exchange mechanism of this important transporter.

a. She J, Guo J, Chen Q, Zeng W, Jiang Y, **Bai XC†**. Structural insights into the voltage and phospholipid activation of the mammalian TPC1 channel. **Nature**. 2018 Apr 5;556(7699):130-134. PMCID**:** PMC5886804 **(†Correspondence author)**

b. Guo J, She J, Zeng W, Chen Q, **Bai XC†**, Jiang Y**†**. Structures of the calcium-activated, non-selective cation channel TRPM4. **Nature**. 2017 Dec 14;552(7684):205-209. PMCID: PMC5901961 **(†Correspondence author)**

c. Wang Y, Nguyen N, She J, Zeng WZ, Yang Y, **Bai XC†**, Jiang Y†. Structural Mechanism of EMRE-Dependent Gating of the Human Mitochondrial Calcium Uniporter. **Cell** 2019. May 16. 177(5): 1252-1261. PMCID: PMC6597010 **(†Correspondence author)**

d. Xue J, Xie T, Zeng W, Jiang Y†, **Bai XC†**. Cryo-EM structures of human ZnT8 in both outward-and inward-facing conformations. **eLife** 2020. PMCID: PMC7428307 **(†Correspondence author)**

5. **Structural and functional studies of receptor tyrosine kinases**: I have established a highly productive research program on systematic structural and functional studies of receptor tyrosine kinases (RTKs), a large family of receptors that are essential for diverse physiological processes. The working mechanisms for several RTKs, which are activated in unique ways, are still poorly understood. Therefore, our understanding of molecular mechanisms underlying activation of RTKs is incomplete. To fill this gap, my lab is currently working on several unique members of RTK, including c-MET, EGFR, RET, IR, IGF1R, TAM and MuSK. Recently, four papers, which report the high-resolution structures of RET, IGF1R, IR, and c-MET respectively, have been published by my lab. Solving the structures of different members of the RTK family will reveal similarities and differences in the structure-function relationships within this receptor family, and pave the way for the comprehensive understanding of how this important receptor

family functions. As a collection, the studies add even greater value in demonstrating the general principle that RTKs can be driven into different structural states by different ligands, which correspond to different signaling states.

a. Li J, Choi E†, Yu HT†, **Bai XC†.** Structural basis of the activation of type 1 insulin-like growth factor receptor. ***Nature Communications***. 2019*.* PMCID: PMC6783537 **(†Correspondence author)**
b. Uchikawa E, Choi E†, Shang GJ, Yu HT†, **Bai XC†**. Activation mechanism of the insulin receptor revealed by cryo-EM structure of the fully liganded receptor–ligand complex. ***eLife.*** 2019*.* PMCID**:** PMC6721835 **(†Correspondence author)**
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