BIOGRAPHICAL SKETCH

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NAME: Xiangdong Zheng

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

POSITION TITLE: Postdoctoral Fellow

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
Harbin Institute of Technology, Weihai, China	B.S.	7/2008	Biochemistry
Tsinghua University, Beijing, China	M.S.	7/2011	Crystallography
Tsinghua University, Beijing, China	Ph.D.	6/2015	Crystallography
Tsinghua University, Beijing, China	Postdoctor	9/2018	Cryo-EM, Epigenetics
Columbia University, New York, NY, US	Postdoctor	present	Cryo-EM, Ion Channel

A. Personal Statement

I have been working on protein structural biology since 2008 when I began my graduate study in School of Medicine at Tsinghua University. My master thesis was on crystal structure of esterase Rv0045c from *Mycobacterium tuberculosis* and my Ph.D. thesis was on structural basis for function of centrosomal protein Sas-4/CPAP in centrosome duplication, through which I obtained extensive training and experience in protein crystallography and became proficient in all procedures in this field, including protein expression, purification and crystallization, X-ray diffraction data collection, phasing, model building and refinement, and structure analysis and presentation. During my Ph.D. training, I sought to illustrate two fundamental questions: how pericentriolar material (PCM) components were tethered, and how centriolar/ciliary-microtubule length was controlled by Sas4/CPAP during centrosome biogenesis. I solved the crystal structure of TCP domain of Sas-4, and the complex structure of CPAP PN2-3 domain bound to alpha/beta-tubulin dimer, which provided structural insights into multi-function of Sas-4/CPAP in centrosome biogenesis.

I began to study cryo-electron microscopy (cryo-EM) in Center for Structural Biology at Tsinghua University in 2015, and meanwhile, did a short period of postdoctoral research there after my Ph.D. training. I worked on the project about cryo-EM architecture of *Saccharomyces cerevisiae* acetyltransferase SAGA complex, through which I learned the technique of single particle analysis (SPA), and accumulated skills and experiences on negative staining, cryogenic specimen-preparation, data acquisition, image processing and 3D reconstruction. I purified endogenous holoenzyme of SAGA complex from *S. cerevisiae*, which is 1.8 MDa and composed of 19 subunits. Using the method of SPA, we solved the cryo-EM structure of SAGA complex at 6.9 Å resolution, with the largest subunit Tra1 resolved at 4.6 Å.

After joining Prof. Yang's lab in Department of Biological Science, Columbia University, I have been mainly focused on structure, function, regulation and disease mechanisms of cyclic nucleotide-gated (CNG) channels, transient receptor potential (TRP) channels and voltage-gated calcium channels (VGCCs), by combining approaches including cryo-EM, biochemistry, cell biology and electrophysiology. My former colleagues have solved the cryo-EM structures of TRPML3 in closed, agonist-activated, and low-pH-inhibited states, and CNG channel in open state. My work is to solve the structure of CNG channel in closed state, and disease-causing mutant structures of TRPML3. So far, I have been able to obtain the wild-type and mutant CNG channel

proteins with good quality and quantity, and will move on to solve the unliganded and mutant structures of CNG channel by cryo-EM.

1. Xu L, Guo J, Zheng X, Wen T, Sun F, Liu S & Pang H. (2010) Crystallization and preliminary X-ray analysis of a novel esterase Rv0045c from *Mycobacterium tuberculosis*. ***Acta Crystallogr Sect F*** 66(Pt 12):1579-1582.
2. Guo J*, Zheng X*, Xu L, Liu Z, Xu K, Li S, Wen T, Liu S & Pang H. (2010) Characterization of a novel esterase Rv0045c from *Mycobacterium tuberculosis*. ***PLoS One*** 5(10):e13143. (*equal contribution)
3. Zheng X*, Guo J*, Xu L, Li H, Zhang D, Zhang K, Sun F, Wen T, Liu S & Pang H. (2011) Crystal structure of a novel esterase Rv0045c from *Mycobacterium tuberculosis*. ***PLoS One*** 6(5):e20506. (*equal contribution)
4. Zheng X*, Gooi LM*, Wason A, Gabriel E, Mehrjardi NZ, Yang Q, Zhang X, Debec A, Basiri ML, Avidor-Reiss T, Pozniakovsky A, Poser I, Saric T, Hyman AA, Li H & Gopalakrishnan J. (2014) Conserved TCP domain of Sas-4/CPAP is essential for pericentriolar material tethering during centrosome biogenesis. ***Proc Natl Acad Sci U S A*** 111(3):E354-363. (*equal contribution)
5. Zheng X*, Ramani* A, Soni K, Gottardo M, Zheng S, Ming Gooi L, Li W, Feng S, Mariappan A, Wason A, Widlund P, Pozniakovsky A, Poser I, Deng H, Ou G, Riparbelli M, Giuliano C, Hyman AA, Sattler M, Gopalakrishnan J & Li H. (2016) Molecular basis for CPAP-tubulin interaction in controlling centriolar and ciliary length. ***Nat Commun*** 7:11874. (*equal contribution)
6. Zheng X*, Liu G*, Guan H*, Cao Y, Kang J, Qu H, Ren X, Lei J, Dong M, Li X & Li H. (2018) Architecture of *Saccharomyces cerevisiae* SAGA complex. Under review in ***Genes Dev***. (*equal contribution)

B. Positions and Honors

Positions and Employment

7/15 - 9/18 Postdoctoral Fellow, Tsinghua University, School of Medicine
9/18 - present Postdoctoral Fellow, Columbia University, Dept. of Biological Sciences

C. Contribution to Science

1. During my master training, I primarily worked on the enzymatic and structural study of *M. tuberculosis* esterase Rv0045c. I contributed to expression, purification and crystallization of esterase Rv0045c (Xu et al., 2010), and determination of optimal catalytic condition and potential substrates for this enzyme (Guo et al., 2010). Beyond the biochemical study, I solved the crystal structure of esterase Rv0045c at 2.8 Å resolution, using the method of molecular replacement (Zheng et al., 2011). Combining docking analysis, I proposed that Rv0045c probably adopts two kinds of enzymatic mechanisms when hydrolyzing C-O ester bonds within substrates (Zheng et al., 2011). Our work provided structural insights into the hydrolysis mechanism of C-O ester bond, and will be helpful for understanding the ester/lipid metabolism in *M. tuberculosis*. I also contributed to crystallization of a novel cationic antimicrobial peptide cecropin B from *Bombyx mori* (Liu et al., 2010).

    a. Xu L, Guo J, Zheng X, Wen T, Sun F, Liu S & Pang H. (2010) Crystallization and preliminary X-ray analysis of a novel esterase Rv0045c from *Mycobacterium tuberculosis*. ***Acta Crystallogr Sect F*** 66(Pt 12):1579-1582.
    b. Guo J*, Zheng X*, Xu L, Liu Z, Xu K, Li S, Wen T, Liu S & Pang H. (2010) Characterization of a novel esterase Rv0045c from *Mycobacterium tuberculosis*. ***PLoS One*** 5(10):e13143. (*equal contribution)
    c. Zheng X*, Guo J*, Xu L, Li H, Zhang D, Zhang K, Sun F, Wen T, Liu S & Pang H. (2011) Crystal structure of a novel esterase Rv0045c from *Mycobacterium tuberculosis*. ***PLoS One*** 6(5):e20506. (*equal contribution)

d. Liu Z, Zhou Q, Mao X, **Zheng X**, Guo J, Zhang F, Wen T & Pang H. (2010) Crystallization and preliminary X-ray analysis of cecropin B from *Bombyx mori*. ***Acta Crystallogr Sect F*** 66(Pt 7):851-853.

2. During my Ph.D. thesis, I worked on the molecular basis for function of Sas-4/CPAP in centrosome duplication. Sas-4/CPAP is one of over hundred centrosomal proteins that contribute to centrosome biogenesis. Two major functions have been explored for Sas-4/CPAP during centrosome biogenesis: one is to scaffold and tether PCM components to newly assembled centrioles, and the other one is to control the length of centriolar and ciliary microtubules. By solving the crystal structure of TCP domain of Sas-4 (Zheng et al., 2014) and the complex structure of CPAP PN2-3 domain bound to alpha/beta-tubulin dimer (Zheng et al., 2016), we are one of the two groups to illustrate the molecular basis for Sas-4 TCP domain in PCM tethering and CPAP-tubulin interaction in centriolar/ciliary-microtubule length control. I performed total internal reflection fluorescence (TIRF) microscopy assay to analyze the PN2-3's bound-tubulin releasing ability and CPAP's microtubule polymerization ability *in vitro*. We are the first to discover two distinct mutations in PN2-3 domain that exhibit opposite effects on centriolar/ciliary-microtubule length (Zheng et al., 2016), which complemented our structural work and enabled us to probe into the molecular mechanism for CPAP-tubulin interaction in controlling centriolar and ciliary length.

   a. Zheng X*, Gooi LM*, Wason A, Gabriel E, Mehrjardi NZ, Yang Q, Zhang X, Debec A, Basiri ML, Avidor-Reiss T, Pozniakovsky A, Poser I, Saric T, Hyman AA, Li H & Gopalakrishnan J. (2014) Conserved TCP domain of Sas-4/CPAP is essential for pericentriolar material tethering during centrosome biogenesis. ***Proc Natl Acad Sci U S A*** 111(3):E354-363. (*equal contribution)
   b. Zheng X*, Ramani* A, Soni K, Gottardo M, Zheng S, Ming Gooi L, Li W, Feng S, Mariappan A, Wason A, Widlund P, Pozniakovsky A, Poser I, Deng H, Ou G, Riparbelli M, Giuliano C, Hyman AA, Sattler M, Gopalakrishnan J & Li H. (2016) Molecular basis for CPAP-tubulin interaction in controlling centriolar and ciliary length. ***Nat Commun*** 7:11874. (*equal contribution)

3. During my postdoctoral research in Center for Structural Biology at Tsinghua University, I mainly focused on cryo-EM architecture of *S. cerevisiae* SAGA complex. SAGA complex is one of the acetyltransferases in yeast and vertebrates, which is about 1.8 MDa and contains at least 19 subunits. The acetyltransferase SAGA plays important role in gene transcription by RNA polymerase II and was determined to be a general coactivator. We purified the intact SAGA holoenzyme from *S. cerevisiae* and solved the cryo-EM structure of SAGA at 6.9 Å resolution using the method of single particle analysis (Zheng et al., 2018). We also resolved the cryo-EM structure of the largest subunit Tra1 at 4.7 Å resolution. Via segmentation analysis facilitated by chemical cross-linking of proteins coupled with mass spectrometry analysis, our work depicted the overall spatial arrangement of SAGA components and provided insights into the molecular architecture and functional model of SAGA complex. I also contributed to the research on histone modifications regulating chromatin compartmentalization via phase separation (Wang et al., 2018). I prepared native H2A, H2B, H3 and H4 histone proteins, K9 tri-methylated H3 histone protein and chromatin template DNA fragments, and reconstituted chromatin fibers containing twelve nucleosome repeats with high quality, which ensured the repeatability, consistence and reliability of all *in vitro* phase separation assays. This work suggests a general mechanism by which histone marks regulate chromosome compartmentalization.

   a. Liu G*, Zheng X*, Guan H*, Cao Y, Kang J, Qu H, Ren X, Lei J, Dong M, Li X & Li H. (2018) Architecture of *Saccharomyces cerevisiae* SAGA complex. Accepted in ***Cell Discov***. (*equal contribution)
   b. Wang L*, Gao Y*, Zheng X*, Liu C, Dong S, Li R, Zhang G, Wei Y, Qu H, Li Y, Allis CD, Li G, Li H & Li P. (2018) Histone modifications regulate chromatin compartmentalization by contributing to a phase separation mechanism. Under revision in ***Mol Cell***. (*equal contribution)

4. I have contributed to structure determination and isothermal titration calorimetry analysis of several epigenetic factors, including histone N-terminal methyltransferase NRMT1 (Wu et al., 2015), transcriptional corepressor ZMYND8 (Li et al., 2016) and methyltransferase SETD2 (Yang et al., 2016).

a. Wu R, Yue Y, Zheng X & Li H. (2015) Molecular basis for histone N-terminal methylation by NRMT1. ***Genes Dev*** 29(22):2337-2342.
b. Li N, Li Y, Lv J, Zheng X, Wen H, Shen H, Zhu G, Chen TY, Dhar SS, Kan PY, Wang Z, Shiekhattar R, Shi X, Lan F, Chen K, Li W, Li H & Lee MG. (2016) ZMYND8 Reads the Dual Histone Mark H3K4me1-H3K14ac to Antagonize the Expression of Metastasis-Linked Genes. ***Mol Cell*** 63(3):470-484.
c. Yang S, Zheng X, Lu C, Li GM, Allis CD & Li H. (2016) Molecular basis for oncohistone H3 recognition by SETD2 methyltransferase. ***Genes Dev*** 30(14):1611-1616.

D. Research Support

None

BIOGRAPHICAL SKETCH

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NAME: Fu, ZIAO

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POSITION TITLE: GRADUATE STUDENT

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
JILIN UNIVERSITY, CHANGCHUN, CHINA	B.S.	2012	CHEMISTRY

A. Personal Statement

I have been working in the cryo-EM field for five years in Joachim Frank's lab as a graduate student. Mainly I am working on time-resolved cryo-EM method development and application. I showed a few successful cases to demonstrate the time-resolved cryo-EM method can capture short-lived intermediates which have half-life time from 10 ms to 1 s. Apart from this main project I am working on, I am also involved in a few other projects to help the researchers to succeed in solving structures using cryo-EM technique. One exciting example is a collaboration with Youzhong Guo and Wayne Hendrickson's lab on membrane protein extraction method development. Avoiding detergent in protein extraction from cell membrane, we can preserve native lipids and we observed for the native lipid bilayer directly extracted from native cell membrane. The method would help researchers working with membrane protein to gain more insight in terms of protein lipid interaction and lipid functional and structure roles.

B. Positions and Honors

Research Assistant 2014-2018 Columbia University.

C. Contributions to Science

1. The structural basis for release factor activation during translation termination revealed by time resolved cryo-EM. 2018 **Ziao Fu*, Gabriele Indrisiunaite*, Sandip Kaledhonkar*, Binita Shah, Ming Sun, Bo Chen, Robert A. Grassucci, Måns Ehrenberg, Joachim Frank** (in review)

   We determined high-resolution structures of short-lived intermediates in the translation termination process using time-resolved Cryo-EM technique.

2. Real-time structural dynamics of late steps in bacterial translation initiation visualized using time-resolved cryogenic electron microscopy. 2018 **Sandip Kaledhonkar* , Ziao Fu* , Kelvin Caban* , Wen Li , Bo Chen , Ming Sun , Ruben Gonzalez Jr, Joachim Frank** （Nature in press）
   We determined high-resolution structures of short-lived intermediates in the translation initiation process using time-resolved Cryo-EM technique.

3. Structure and Activity of Lipid Bilayer within a Membrane Protein Transporter 2018 **Weihua Qiu*, Ziao Fu*, Guoyan G. Xu, Robert A. Grassucci, Yan Zhang, Joachim Frank, Wayne A. Hendrickson, Youzhong Guo** Proc Natl Acad Sci U S A. 2018 Dec 18;115(51):12985-12990.
   We solved native lipid bilayer structure by Cryo-EM technique at high resolution about 3 A.

4. A Fast and Effective Microfluidic Spraying-Plunging Method for High-Resolution Single-Particle Cryo-EM 2017 **Xiangsong Feng*, Ziao Fu*, Sandip Kaledhonkar, Yuan Jia, Binita Shah, Amy Jin, Zheng Liu, Ming Sun, Bo Chen, Robert A Grassucci, Yukun Ren, Hongyuan Jiang, Joachim Frank, Qiao Lin** Structure 25 (4), 663-670. e3
   We describe a spraying-plunging method for preparing cryoelectron microscopy (cryo-EM) grids with vitreous ice of controllable, highly consistent thickness using a microfluidic device. The new polydimethylsiloxane (PDMS)-based sprayer was tested with apoferritin. We demonstrate that the structure can be solved to high resolution with this method of sample preparation. Besides replacing the conventional pipetting-blotting-plunging method, one of many potential applications of the new sprayer is in time-resolved cryo-EM, as part of a PDMS-based microfluidic reaction channel to study short-lived intermediates on the timescale of 10-1,000 ms.

5. Key intermediates in ribosome recycling visualized by time-resolved cryoelectron microscopy 2016 **Ziao Fu*, Sandip Kaledhonkar*, Anneli Borg*, Ming Sun, Bo Chen, Robert A Grassucci, Måns Ehrenberg, Joachim Frank** Structure 24 (12), 2092-2101

   We determined the structures of short-lived intermediates in the translation recycling process using time-resolved cryo-EM technique. Upon encountering a stop codon on mRNA, polypeptide synthesis on the ribosome is terminated by release factors, and the ribosome complex, still bound with mRNA and P-site-bound tRNA (post-termination complex, PostTC), is split into ribosomal subunits, ready for a new round of translational initiation. Separation of post-termination ribosomes into subunits, or “ribosome recycling,” is promoted by the joint action of ribosome-recycling factor (RRF) and elongation factor G (EF-G) in a guanosine triphosphate (GTP) hydrolysis-dependent manner. Here we used a mixing-spraying-based method of time-resolved cryo-electron microscopy (cryo-EM) to visualize the short-lived intermediates of the recycling process. The two complexes that contain (1) both RRF and EF-G bound to the PostTC or (2) deacylated tRNA bound to the 30S subunit are of particular interest. Our observations of the native form of these complexes demonstrate the strong potential of time-resolved cryo-EM for visualizing previously unobservable transient structures.

D. Additional Information: Research Support and/or Scholastic Performance

Not applicable.

I am supported by this grant. But I don’t know if I should list it here or how to describe my role in the grant.

Ongoing

RO1 GM029169 JOACHIM FRANK, PI 1994 – 2019

NIH NIGMS
STRUCTURAL ANALYSIS OF MACROMOLECULAR ASSEMBLIES
This study explores structure and function of the ribosome actively engaged in protein synthesis, by cryo-electron microscopy (cryo-EM) and single-particle reconstruction.

BIOGRAPHICAL SKETCH

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NAME: Jian Yang

eRA COMMONS USER NAME (credential, e.g., agency login): jianyang160

POSITION TITLE: Professor of Biological Sciences

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
Peking University, Beijing, China	B.S.	06/1982	Biophysics
Shanghai Brain Research Institute, China	M.S.	07/1985	Neurophysiology
University of Washington, Seattle, WA	Ph.D.	06/1991	Physiol. & Biophy.
Stanford University, Stanford, CA	Postdoctor	12/1993	Ca^{2+} Channels
UCSF, San Francisco, CA	Postdoctor	12/1996	K^+ Channels

A. Personal Statement

I have been working on ion channels since graduate school. For my PhD thesis in Bertil Hille's lab, I characterized the biophysical properties of the 5-HT3 receptor channel. My postdoc work in Dick Tsien's lab was focused on figuring out "what makes a calcium channel a calcium channel", as we used to say in the Tsien lab. During my second postdoc in Lily Jan's lab, I learned much more ion channel molecular biology and worked on the subunit stoichiometry of inward rectifier potassium (Kir) channels and the molecular determinants of inward rectification and ion permeation. After establishing my own lab at Columbia in 1997, I initially continued to work on Kir channels, primarily by using mutagenesis and patch clamp. In 2000, I returned to work on voltage-gated calcium channels (VGCCs), and in 2004, we also started to work on TRP channels. In 2002, spurred by the spectacular crystallographical studies of Rod MacKinnon on potassium channels, I decided to do X-ray crystallography in my own lab, which we have been doing ever since. In 2014, we began to do cryo-EM, this time inspired by the stunning success of the TRPV1 cryo-EM structure obtained by David Julius and Yifan Cheng and frustrated by many years of failure to get a crystal structure of a full-length channel. I consider these career moves important, timely, exciting and rewarding. In 2011, in collaboration with the Kunming Institute of Zoology (KIZ) of the Chinese Academy of Sciences, and with the support of my department, I set up an Ion Channel Research and Drug Development Center (ICDC) at KIZ, with the main goal of discovering natural products of therapeutic potential and/or as research tools that target ion channels. I work there on a part-time basis as a visiting investigator. No NIH funds have been or will be used at ICDC.

My research focuses on the structure, function, regulation, disease mechanisms and drug discovery of calcium-conducting channels, including VGCCs, TRP channels and cyclic nucleotide-gated (CNG) channels. We strive to better understand how these channels work as molecular machines and how they control and regulate diverse physiological and pathological processes. Our past work touched upon the pore architecture of VGCCs, the location of the activation gate, the crystal structure of VGCC β subunits, the identification of novel $Ca_V\beta$ interacting proteins, the molecular mechanisms of regulation of VGCCs by PIP_2, G proteins, RGK proteins and proteolysis, the molecular mechanisms of the assembly of TRPP/PKD complexes, the structural basis of regulation and function of TRPML channels, and the cryo-EM structure of a full-length eukaryotic CNG channel.

We use various approaches in our research, including molecular biology, biochemistry, cell biology, electrophysiology, calcium imaging, confocal microscopy, X-ray crystallography and cryo-EM. We have the

necessary motivation, expertise, tools and collaboration to carry out the proposed projects. This is further demonstrated by the large amount of preliminary data we have gathered for this application. I have 32 years of research experience (starting when I was a M.S. student). I have had continuous NIH grant support since the establishment of my own lab, and I am the PI of two ongoing and seven completed RO1s. Thus, I have the required leadership skill and experience in organizing, executing and completing research projects.

1. Li, M.*, Zhou, X.*, Wang, S.*, Michailidis, I.E., Gong, Y., Su, D., Li, H., Li, X.#, and **Yang, J.#** (2017). Structure of a eukaryotic cyclic nucleotide-gated channel. **Nature** 542, 60-65.
2. Michailidis, I.E., Abele, K., Zhang, W.K., Lin, B., Yu, Y., Geyman, L., Ehlers, M.D., Pnevmatikakis, E.A., and **Yang J.** (2014). Age-related homeostatic midchannel proteolysis of L-type voltage-gated Ca^{2+} channels. ***Neuron*** 82, 1045-1057. (PMCID: PMC4052215)
3. Wu, L.*, Bauer, C*., Zhen, X-G., Xie, C., and **Yang, J.** (2002). Dual regulation of voltage-gated calcium channels by PIP_2. ***Nature*** 419, 947-952.
4. Zhou, X.*, Li, M-H.*, Su, D.*, Li, H., Jia, Q., Li, X.#, and **Yang, J.#** (2017). Cryo-EM structures of the human endolysosomal TRPML3 channel in three distinct states. **Nat. Struc. Mol. Biol.** 24, 1146-1154. (PMC5747366)

B. Positions and Honors

Positions and Employment

1985-1987	Visiting Scholar, Colorado State University, Dept. of Neurobiology & Anatomy, Ft. Collins, CO
1997-2002	Assistant Professor, Columbia University, Dept. of Biological Sciences, New York, NY
2002-2009	Associate Professor, Columbia University, Dept. of Biological Sciences, New York, NY
2009-	Professor, Columbia University, Dept. of Biological Sciences, New York, NY
2011-	Visiting Investigator, Ion Channel Research and Drug Development Center, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China

Other Experience and Professional Memberships

1997-	Member, Biophysical Society
2004-2008	NIH NTRC Study Section, Regular member
2004-2010	Editorial Board, Biophysical Journal
2014-	Editorial Board, Zoological Research (Kunming, China)
2015-	Editorial Board, Journal of Physiology (London)
2015-	Editorial Board, Channels (Canada)

Honors

1997-1999	Sloan Research Fellow, Alfred P. Sloan Foundation
2000-2003	McKnight Scholar Award, The McKnight Endowment Fund for Neuroscience
2002-2004	EJLB Scholar, The EJLB Foundation
2004-2008	Established Investigator Award, The American Heart Association

C. Contribution to Science

1. My early work as an independent junior PI centered on inward rectifier potassium (Kir) channels. It was an exciting time to work on potassium channels, especially after the publication of the first crystal structure of an ion channel by Rod MacKinnon. Compared to what was known about voltage-gated potassium channels, much less was known about the assembly, pore architecture and location of the activate gate in Kir channels. Using cysteine chemical modification, we discovered that the Kir channel pore is 12 Å wide (Lu et al., 1999a), demonstrating that the inner pore of an open K^+ channel is much wider than what is shown at the time by the crystal structure of the KcsA K^+ channel. We also demonstrated that the cytoplasmic domains of Kir channels form a long and wide intracellular vestibule that protrudes beyond the membrane into the cytoplasm (Lu et al., 1999b), a finding later confirmed by crystal structures of Kir channels obtained by other laboratories. Moreover, using the cutting-edge technology of unnatural amino acid mutagenesis, we engineered artificial amino acids into Kir channels and demonstrated directly that the K^+ selectivity filter

is dynamic and controls Kir channel gating (Lu et al., 2001). This work indicates that the selectivity filter of Kir channels can function as a gate, a conclusion further supported by our later work showing the lack of state-dependent modification of cysteines residues engineered below the selectivity filter by intracellular thiol-specific reagents (Xiao et al., 2003).

a. Lu, T., Zhang, X-M., Nguyen, B., and **Yang, J.** (1999). Architecture of a K^+ channel inner pore revealed by stoichiometric covalent modification. ***Neuron*** 22, 571-580.
b. Lu, T., Zhu, Y-G., and **Yang, J.** (1999). Cytoplasmic amino and carboxyl domains form a wide internal vestibule in an inwardly rectifying K^+ channel. ***Proc. Natl. Acad. Sci.*** 96, 9926-9931.
c. Lu, T., Ting, A.Y., Mainland, J., Jan, L.Y., Schultz, P.G., and **Yang, J**. (2001). Probing ion permeation and gating in a K^+ channel with backbone mutations in the selectivity filter. ***Nature Neurosci.*** 4, 239-246.
d. Xiao, J., Zhen, X-G., and **Yang, J.** (2003). Localization of PIP_2 activation gate in inward rectifier K^+ channels. ***Nature Neurosci.*** 6, 811-818.

2. We have made three major discoveries in the study of voltage-gated calcium channels (VGCCs): (1) We are the first to discover that VGCCs are regulated by PIP_2 (Wu et al., 2002), providing mechanistic insights into the regulation of VGCCs by Gq-coupled receptors; (2) We are one of the three groups that simultaneously solved the first crystal structure of the beta subunit of VGCCs (Chen et al., 2004), which is essential for trafficking the channel complex to the plasma membrane and fine-tuning channel biophysical properties. The structure overturns a then widely accepted and long-held doctrine regarding where and how the alpha 1 and beta subunits interact; (3) We recently discovered that the alpha 1 subunit of neuronal L-type VGCCs undergoes a novel form of age- and activity-dependent proteolysis (called midchannel proteolysis) in the pore-forming core region (Michailidis et al., 2014), providing novel molecular insights into neuronal calcium homeostasis and neuroprotection. Each of these discoveries leads to new concepts and new research areas.

a. Wu, L.*, Bauer, C*., Zhen, X-G., Xie, C., and **Yang, J.** (2002). Dual regulation of voltage-gated calcium channels by PIP_2. ***Nature*** 419, 947-952.
b. Chen, Y-h., Li, M-h., Zhang, Y., He., L-l., Yamada, Y., Fitzmaurice, A., Shen, Y., Zhang, H., Tong, L., and **Yang, J.** (2004). Structural basis of the α_1–β interaction of voltage-gated Ca^{2+} channels. ***Nature*** 429, 675-680.
c. Michailidis, I.E., Abele, K., Zhang, W.K., Lin, B., Yu, Y., Geyman, L., Ehlers, M.D., Pnevmatikakis, E.A., and **Yang J.** (2014). Age-related homeostatic midchannel proteolysis of L-type voltage-gated Ca^{2+} channels. ***Neuron*** 82, 1045-1057. (PMCID: PMC4052215)

3. In recent years, we have made significant contributions to the understanding of the structure and function of TRPP/PKD complexes. These ion channel/receptor complexes play critical roles in calcium signaling in cells. They are relatively new, and much is unknown about them. Mutations in these complexes cause human diseases, such as autosomal dominant polycystic kidney disease (ADPKD), one of the most common genetic diseases in humans. Using a multipronged approach that includes biochemistry, electrophysiology, single molecule optical imaging, X-ray crystallography and computational modeling, we have elucidated the molecular mechanisms of the assembly of the TRPP2/PKD1 and TRPP3/PKD1L3 complexes (Yu et al., 2009; Jiang et al., 2011; Yu et al., 2012). A prevailing view in the PKD field was that PKD proteins are membrane receptors, not ion channels, and that they play a regulatory role in TRPP/PKD complexes. Our work indicates that PKD1L3 is in fact a channel-forming protein, directly lining the pore of the TRPP3/PKD1L3 complex (Yu et al., 2012). Our studies have significant implications for the regulation and function of TRPP/PKD complexes and for the pathogenic mechanisms of ADPKD.

a. Yu, Y., Ulbrich, M.H., Li, M-h., Chen, X-Z., Ong, A.C.M., Tong, L., Isacoff, E.Y., and **Yang, J.** (2009). Structural and molecular basis of the assembly of the TRPP2/PKD1 complex. ***Proc. Natl. Acad. Sci.*** 106, 11558-11563. (PMCID: PMC2710685)
b. Zhu, J.*, Yu, Y.*, Ulbrich, M.H., Li, M-h., Isacoff, E.Y., Honig, B., and **Yang, J**. (2011). A structural model of the TRPP2/PKD1 C-terminal coiled-coil complex produced by a combined computational and experimental approach. ***Proc. Natl. Acad. Sci.*** 108, 10133-10138. (PMCID: PMC3121833)

c. Yu, Y., Ulbrich, M.H., Dobbins, S. Li, M-h., Zhang, W.K., Tong, L., Isacoff, E.Y., and **Yang, J.** (2012). Molecular mechanism of the assembly of an acid-sensing receptor/ion channel complex. ***Nat. Commun.*** 3:1252. doi: 10.1038/ncomms2257. (PMCID: PMC3575195)

4. We recently obtained a 3.5 Å-resolution cryo-EM structure of a full length eukaryotic cyclic nucleotide-gated (CNG) channel (Li et al., 2017a). This is the first high-resolution structure of this distinct subfamily of ion channels. The structure reveals some unusual and unique features that have not been seen in any other ion channel structures, including a strikingly different S4 segment. This structure provides insights into CNG channel ion permeation, gating and channelopathy. In recent years we have also been working on the structure, function and regulation of TRPML1 and TRPML3 channels. These channels function as calcium channels in endosomes and lysosomes and are crucial for cellular physiology. Mutations in TRPML1 cause mucolipidosis type IV, a rare but devastating lysosomal storage disorder in humans, and mutations in TRPML3 cause deafness and pigmentation defects in mice. We have determined high-resolution structures of a functionally important luminal domain of TRPML1 (Li et al., 2017b) and the full length TRPML3 (Zhou et al., 2017) under various pH conditions or in different states.
    a. Li, M-h.*, Zhou, X.*, Wang, S.*, Michailidis, I.E., Gong, Y., Su, D., Li, H., Li, X.[#], and **Yang, J.**[#] (2017a). Structure of a eukaryotic cyclic nucleotide-gated channel. ***Nature*** 542, 60-65.(PMCID: PMC5783306)
    b. Li, M-h.*, Zhang, W,K.*, Benvin, N*., Zhou, X., Su, D., Wang, S., Michailidis, I.E., Tong, L., Li, X.[#], and **Yang, J**.[#] (2017b). Structural basis of Ca^{2+}/pH dual regulation of the endolysosomal Ca^{2+} channel TRPML1. **Nat. Struc. Mol. Biol.** 24, 205-213. (PMCID: PMC5336481)
    c. Zhou, X.*, Li, M-h.*, Su, D.*, Li, H., Jia, Q., Li, X.[#], and **Yang, J.**[#] (2017). Cryo-EM structures of the human endolysosomal TRPML3 channel in three distinct states. **Nat. Struc. Mol. Biol.** 24, 1146-1154. (PMC5747366)

5. Complete List of Published Work as PI in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/jian.yang.1/bibliography/41158391/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

RO1 GM085234 Yang (PI) 05/01/15-02/28/19
TRPML1 channel physiology and pathophysiology
The goal of this study is to elucidate how TRPML1 works as molecular machines, its role in lysosomal physiology, and the pathogenic mechanisms of disease causing mutations.
Role: PI

R01 EY027800-01 Yang (PI) 04/01/17-03/31/20
Molecular physiology of cyclic nucleotide-gated channels
The goal of this study is to obtain structures of full length eukaryotic cyclic nucleotide-gated (CNG) channels in the open and closed states and carry out structure-based functional studies to understand how CNG channels work as molecular machines.
Role: PI

Completed Research Support

R01 NS 053494 Yang (PI) 12/01/15-4/30/16
Molecular physiology of voltage-gated Ca^{2+} channels
The major goal of this project is to study the signaling molecules and pathways, the dynamics, and the functional consequences of a novel form of proteolysis of the pore-forming alpha 1 subunit of voltage-gated Ca^{2+} channels.
Role: PI