BIOGRAPHICAL SKETCH

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NAME: Sunjae Park

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

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
Kyungpook National University, Daegu, Korea	B.S.	5/2014	Chemistry
Stony Brook University, Stony Brook, NY, US	M.S.	8/2018	Biochemistry and Cell Biology

A. Personal Statement

I have been working on ion channel after I joined Dr. Jian Yang's lab as a graduate student. My ongoing project is on structure and regulation mechanisms of transient receptor potential (TRP) channels 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. My work is to solve the structure of TRPML2 channel in closed, agonist-activated, and low-pH-inhibited states. I have not been in the structural field for long time but I have already showed a few successful results. We were able to purify both the apo and agonist-bound TRPML2 proteins with good quality and quantity, and obtain a 3. 37 Å structure of apo TRPML2. With the structural approach and functional study methods which I am learning from my project, I will move on to solve the agonist-bound structures of TRPML2 by cryo-EM and understand the regulation mechanism of TRPML2.

B. Positions and Honors

7/17 - 8/18 Research assistant, Stony Brook University
9/18 - present Graduate student, Columbia University, Dept. of Biological Sciences

C. Contribution to Science

1. During my master study, I worked in Dr. Erwin London's lab as a research assistant. I started the project of studying properties of αCD and γCD to see how their different ring sizes affect the interaction with various phospholipids with different acyl chain structures. Cyclodextrins (CD) are cyclic oligosaccharides and they can be comprised of six, seven, or eight α-(1,4)-glucopyranose units, termed α-, β-, or γ-cyclodextrins, respectively. CDs have ability to encapsulate hydrophobic or amphiphilic molecules by having a hydrophilic surface and a hydrophobic cavity. CDs have numerous applications by improving handling of volatile or poorly water-soluble substances and improving bioavailability. CDs are also used in investigating natural cell membranes. Compared to modified CD, not much research has been carried out on exchange using non-modified CDs especially with αCD and γCD. We have carried out studies using αCD and γCD in cyclodextrin-mediated lipid vesicle solubilization and lipid exchange. Furthermore, I investigated the ability of αCD and γCD in preparing asymmetric artificial vesicles using FRET and HP-TLC. We found that αCD may not be suitable for preparation of asymmetric vesicles but γCD has capacity to exchange lipids to prepare asymmetric vesicles and is worthy for further investigation.

2. In Dr. Jian Yang's lab, I am working on structural basis of activation and regulation of an endolysosomal channel TRPML2. TRPMLs are endolysosomal non-selective cation channels and known to have roles in endosome-lysosome fusion, vacuolar pH regulation, exocytosis, metal homeostasis, and autophagy. Recent studies suggest that TRPML2 is crucial for immune cell processes. With the structural approach, we were able to obtain the structure of TRPML2 in apo state, and I will move on to determine the structures of agonist-activated TRPML2.

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

None

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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 5-HT3 receptor channels. 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 approval and 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 collaborate with ICDC as a visiting investigator with strict adherence to NIH and Columbia University policies. No NIH funds have been or will be used at ICDC, and no grant at ICDC overlaps with my past and present NIH-sponsored projects.

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 structure, function and disease mechanisms of CNG channels. 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 over 38 years of research experience (starting when I was a M.S. student). I have had continuous NIH grant support since establishing my own lab, and I am the PI of an ongoing and eight completed RO1s. Thus, I have the required leadership skill and experience in organizing, executing and completing research projects.

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

RO1 GM085234
Yang (PI)
09/04/19-08/31/23
Molecular physiology of TRPML channels

R01 EY027800
Yang (PI)
04/01/17-03/31/20
Molecular physiology of cyclic nucleotide-gated channels

Citations: (* indicates co-first authors; #indicates co-corresponding authors)

1. Zheng, X.*, Fu, Z.*, Su, D.*, Zhang Y., Li, M., Pan, Y., Li, H., Li, S., Grassucci, R.A., Ren, Z, Hu, Z., Li, X., Zhou, M., Li, G.[#], Frank, J.[#], and **Yang, J.**[#] (2020). Mechanism of ligand activation of a eukaryotic cyclic nucleotide-gated channel. ***Nat. Struc. Mol. Biol.*** 27, 625-634. (PMCID: PMC7354226)
2. Li, M-h.*, 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. (PMCID: PMC5783306)
3. 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)
4. 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. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2015 – present	Editorial Board, Journal of Physiology (London)
2015 – present	Editorial Board, Channels (Canada)
2015	NIH NTRC Study Section, ad hoc reviewer
2014 – 2017	Editorial Board, Zoological Research (Kunming, China)
2011 – present	Visiting Investigator, Ion Channel Research and Drug Development Center, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China
2011 – 2016	Director, Ion Channel Research and Drug Development Center, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China
2009 – present	Professor, Columbia University, Dept. of Biological Sciences, New York, NY
2004 – 2008	NIH NTRC Study Section, Regular member
2004 – 2010	Editorial Board, Biophysical Journal
2004	NIH NTRC Study Section, ad hoc reviewer
2003	NIH NTRC Study Section, ad hoc reviewer
2002 – 2009	Associate Professor, Columbia University, Dept. of Biological Sciences, New York, NY
1997 – 2002	Assistant Professor, Columbia University, Dept. of Biological Sciences, New York, NY
1985 – 1987	Visiting Scholar, Colorado State University, Dept. of Neurobiology & Anatomy, Ft. Collins, CO

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). 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 regulates 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 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. In 2017 we obtained a 3.5 Å-resolution cryo-EM structure of a full-length eukaryotic cyclic nucleotide-gated (CNG) channel (Li et al., 2017a). This was the first high-resolution structure of this distinct subfamily of ion channels. We have recently obtained high-resolution structures of both closed and open states of the same CNG channel (Zheng et al., 2020). These structures provide 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. (PMCID: PMC5747366)
d. Zheng, X.*, Fu, Z.*, Su, D.*, Zhang Y., Li, M., Pan, Y., Li, H., Li, S., Grassucci, R.A., Ren, Z, Hu, Z., Li, X., Zhou, M., Li, G.[#], Frank, J.[#], and **Yang, J.**[#] (2020). Mechanism of ligand activation of a eukaryotic cyclic nucleotide-gated channel. ***Nat. Struc. Mol. Biol.*** 27, 625-634. (PMCID: PMC7354226)

Complete List of Published Work in MyBibliography:

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