OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

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NAME: Zhengshan Hu

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

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
University of California, Los Angeles	B.S.	03/2016	Microbiology, Immunology, and Molecular Genetics

A. Personal Statement

I have been working on ion channel after I joined Dr. Jian Yang's lab as a graduate student. My first ongoing project is on the regulation of P/Q-type voltage gated calcium channel, a process called mid-channel proteolysis, discovered by our lab. As a second project of my PhD study, I started to work on cyclic nucleotide-gated (CNG) channel structures using cryo-EM. I have not been in the structural field that long but I have already showed a few success purifying both native and mutant CNG channels and was able to get a 3.0 Å structure. With the structural approach and functional study methods which I mastered from my first project such as single channel electrophysiology, I am excited to study the ligand binding cooperativity, disease causing mutation pathology, and the Ca^{2+} block mechanism of CNG channels.

B. Positions and Honors

09/2014 – 03/2016 Research assistant, University of California Los Angeles
09/2016 – present Graduate student, Columbia University

C. Contributions to Science

1. **During my undergraduate study, I worked in Dr. Karen Lyons' lab as a research assistant. I started the project of discovering the role of protein CCN1 in regulation of tendon development. I used mice as model system. I used various techniques such as histology, q-PCR, immunohistochemistry staining, μCT, and genetic knockout by Cre-LoxP system. I found out that on the tissue level, CCN1 gene is vastly expressed in the Achilles tendon and the anterior cruciate ligament (ACL) tendon. It is very important to organize tendon fibers and suppress their overgrowth which will lead to dislocation of knee cap and various of other phenotypes. On the cellular level, I isolated tenocytes from mice Achilles tendons and discovered that increased tension on the cell surface will increase the expression level of CCN1 mRNA.**

   Jiang J, **Hu Z**, Lyons KM. Design and Analysis of CCN Gene Activity Using CCN Knockout Mice Containing LacZ Reporters. Methods Mol Biol. 2017;1489:325–345. doi: 10.1007/978-1-4939-6430-7_28.

2. **In Dr. Jian Yang's lab, I am working on mid-channel proteolysis of P/Q-type voltage-gated calcium channel. We found out in both mice cerebellum and cultured neurons, P/Q-type**

channels could be cleaved spontaneously in the middle of the pore-forming core region into two parts. I used P/Q-type antibodies to isolate the second half of the channel and discovered some potential cleavage sites and will use mutant channels to verify the sites. I will move on to determine the cleavage mechanism and physiological significance.

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

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 $\alpha_1-\beta$ 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