BIOGRAPHICAL SKETCH

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

NAME: Clarke, Oliver B

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

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Melbourne	BSc(Hons)	11/2007	Chemistry & Biochemistry
University of Melbourne (Walter & Eliza Hall Institute of Medical Research)	PhD	07/2011	Structural Biology
Columbia University	Posdoctoral training	09/2017	Structural Biology

A. Personal Statement

As an Assistant Professor at Columbia University, I am well positioned to advance the proposed program of research. Broadly, my research as a postodoctoral scientist in the Hendrickson laboratory, and now in my capacity as an independent investigator has focused on the structural analysis of membrane proteins by single particle cryoelectron microscopy (cryoEM), with a particular focus on ion channel gating and activation. I am currently PI on an NIH R01 grant aimed at understanding the structural basis of regulation of the skeletal muscle ryanodine receptor by ligands and protein binding partners, and also serve as CoI on a number of other NIH-funded grants. My laboratory has also been engaged in work to understand the molecular architecture of the red blood cell membrane, with a particular focus on the ankyrin complex, which clusters key membrane proteins involved in membrane transport and links the membrane to the cytoskeleton.

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

R01AR077720 04/01/21-03/31/22
Structural basis for allosteric regulation of RyR1
The goal of this proposal is to understand how small molecules and protein binding partners regulate RyR1 activity by binding to peripheral sites, using a combination of cryoEM and functional approaches.
Role: PI

R01 NS109366-01A1 08/15/19-06/30/24
Structural studies of HCN channels in health and disease
This project is aimed at understanding the structural basis of gating in the HCN4 channel, and regulation of gating by accessory proteins.
Role: CoI

R01 HL145473 08/23/19-04/30/23
Structure-function analysis for elucidating pathogenicity of cardiac ryanodine receptor genetic variants
This project is aimed at understanding using structure/function approaches how pathogenic mutations in RyR2 lead to channel activation.
Role: CoI

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2017- present Assistant Professor, Columbia University, Department of Anesthesiology (Secondary appointments in Physiology & Cellular Biophysics and the Irving Institute for Clinical and Translational Research)
2012-2017 Postdoctoral Fellow, Columbia University, Dept. Biochem & Mol. Biophys.

Honors

2014 Charles H. Revson Senior Fellowship
2012 Overseas Biomedical Fellowship (NHMRC, Australia)

C. Contributions to Science

1. *Ion channel architecture and gating*. During my graduate work, I described the structure of multiple conformational states of a bacterial inwardly rectifying postassium channel, KirBac 3.1, determined by X-ray crystallography. This work led to an ongoing fascination with the structure and activation and gating mechanisms of ion channels, which I further explored during my postdoctoral work in the laboratory of Wayne Hendrickson, which entailed solving the structure of the ryanodine receptor RyR1, an intracellular calcium release channel of exceptional size and complexity. This project, initially a collaboration between the Hendrickson lab and that of Andrew Marks, was initially focused on the use of X-ray crystallography to solve the structure of the channel, but obtaining well-diffracting crystals proved difficult. As a result, I switched techniques, initiating a collaboration with the laboratory of Joachim Frank, which resulted in a reconstruction of the closed, ligand-free state of the receptor at 4.8 Å, and later the structures of multiple ligand-bound conformations including open states at resolutions up to 3.7 Å. This work allowed me to gain experience in cryoEM data collection, processing, and the building and refinement of macromolecular models into EM maps. I have continued structural studies of the skeletal muscle ryanodine receptor in my own laboratory, with a focus on understanding how ligands and binding partners binding in peripheral regions of the receptor allosterically regulate channel gating. In recent, unpublished work, we have solved cryoEM structures of the receptor at resolutions up to 2.1Å in complex with the malignant hyperthermia therapeutic dantrolene, unexpectedly showing that dantrolene binds to the first tandem repeat (RY12) domain of RyR1 in concert with adenine nucleotides. In follow up work we determined that this site is a novel binding site for ATP and ADP, even in the absence of dantrolene, and we are currently investigating the functional and physiological significance of this site.

a. **Clarke OB**, Caputo AT, Hill AP, Vandenberg JI, Smith BJ, Gulbis JM. Domain reorientation and rotation of an intracellular assembly regulate conduction in Kir potassium channels. Cell. 2010; 141(6):1018-29. PMID: 20564790

b. Zalk R*, **Clarke OB***, des Georges A*, Grassucci RA, Reiken S, Mancia F, Hendrickson WA, Frank J, Marks AR. Structure of a mammalian ryanodine receptor. Nature. 2015; 517(7532):44-9. PMCID: PMC4300236

c. des Georges A*, **Clarke OB***, Zalk R*, Yuan Q, Condon KJ, Grassucci RA, Hendrickson WA, Marks AR, Frank J. Structural Basis for Gating and Activation of RyR1. Cell. 2016; 167(1):145-157.e17. PMCID: PMC5142848

d. Melville Z, Kim K, **Clarke OB**, Marks AR. High-resolution structure of the membrane-embedded skeletal muscle ryanodine receptor. Structure. 2022 Jan 6;30(1):172-180.e3. PMCID: PMC8741649

2. *Structure and conformational dynamics of class C GPCRs*. In collaborative effort lead by Qing Fan and her team, we have been involved in structural studies of two class C GPCRs, the $GABA_B$ receptor and the Calcium

sensing receptor (CaSR). Unlike GPCRs from better characterized groups such as the class A receptors, which have ligand binding sites within the classical 7TM bundle, class C GPCRs sense signals via ligand binding to a large extracellular domain. Until recently, there has been little structural information available for intact class C receptors, as the flexible hinge between the transmembrane region and the ligand binding domains makes them recalcitrant to crystallization, and difficult targets even for single particle cryoEM. In our initial work, we have solved the structure of the heterodimeric human $GABA_B$ receptor by cryoEM, in an inactive state. The structure reveals the binding site of multiple ligands of the receptor, including the unexpected presence of native lipids in a transmembrane cleft. Recently, we solved the structure of a second class C GPCR, the homodimeric CaSR, in both active and inactive states, giving insights into the mechanism of receptor activation and modulation by ligands. Much of the cryoEM data processing was performed in my laboratory, and I am co-corresponding author on both of the published works.

a. Park J, Fu Z, Frangaj A, Liu J, Mosyak L, Shen T, Slavkovich VN, Ray KM, Taura J, Cao B, Geng Y, Zuo H, Kou Y, Grassucci R, Chen S, Liu Z, Lin X, Williams JP, Rice WJ, Eng ET, Huang RK, Soni RK, Kloss B, Yu Z, Javitch JA, Hendrickson WA, Slesinger PA, Quick M, Graziano J, Yu H, Fiehn O, **Clarke OB**[*], Frank J[*], Fan QR[*]. Structure of human $GABA_B$ receptor in an inactive state. Nature. 2020 Aug;584(7820):304-309. PMCID: PMC7725281

b. Park J, Zuo H, Frangaj A, Fu Z, Yen LY, Zhang Z, Mosyak L, Slavkovich VN, Liu J, Ray KM, Cao B, Vallese F, Geng Y, Chen S, Grassucci R, Dandey VP, Tan YZ, Eng E, Lee Y, Kloss B, Liu Z, Hendrickson WA[*], Potter CS, Carragher B, Graziano J, Conigrave AD[*], Frank J[*], **Clarke OB**[*], Fan QR[*]. Symmetric activation and modulation of the human calcium-sensing receptor. Proc Natl Acad Sci U S A. 2021 Dec 21;118(51) PMID: 34916296

3. *Molecular architecture of the red blood cell membrane*. In a new direction for my laboratory, we are investigating the molecular architecture of the red blood cell membrane, as it represents both a tractable model system for understanding the organization and architecture of eukaryotic plasma membranes in general, and is also of substantial physiological and biomedical significance in its own right. We are taking a top-down approach to this problem, involving differential solubilization of the membrane using mild detergents, followed by density gradient centrifugation and size exclusion chromatography to separate out large membrane protein complexes. In initial results, we have solved the structure of the 1.2MDa erythrocyte ankyrin complex, which acts to mechanically stabilize the membrane by linking the spectrin-actin cytoskeleton to the membrane, as well as to cluster key membrane proteins involved in ion transport, pH regulation and regulation of cell shape and volume. This work is currently under review at *Nature Structural & Molecular Biology*.

4. *Structure of mammalian thyroglobulin.* Thyroglobulin is a large, flexible, homodimeric protein that acts as both scaffold and substrate for the synthesis of the thyroid hormones, thyroxine and triiodothyronine, as well as serving as a major site of iodine storage. Until recently, no structural information was available concerning the architecture of the protein or the mechanism of hormonogenesis. In order to address this gap in knowledge, we sought to use single particle cryo-EM to solve the structure of bovine thyroglobulin, in collaboration with a leading laboratory in the field, the group of Peter Arvan at the University of Michigan. We solved the structure of bovine thyroglobulin in a natively iodinated state at 2.5Å, identifying two key acceptor-donor tyrosine pairs, and visualizing the bound hormone in the context of the thyroglobulin structure.

a. Kim K, Kopylov M, Bobe D, Kelley K, Eng ET, Arvan P, **Clarke OB**. The structure of natively iodinated bovine thyroglobulin. Acta Crystallogr D Struct Biol. 2021 Nov 1;77(Pt 11):1451-1459.

5. *Structure of cytochrome bo_3.* After accidentally purified E. coli cytochrome bo_3 ubiquinol oxidase and reconstituting it into lipid nanodiscs, while attempting to express another membrane protein of interest, we solved the structure of this important respiratory enzyme to 2.2 Å by cryo-EM, revealing the arrangement of metal redox centers and the binding mode of the ubqiquinone-8 substrate. This work which was conducted in collaboration with the Gennis, Zhang, Zhu and Tajkhorshid laboratories was published earlier this year in *PNAS*. I am co-corresponding author on this publication.

a. Li J, Han L, Vallese F, Ding Z, Choi SK, Hong S, Luo Y, Liu B, Chan CK, Tajkhorshid E, Zhu J[*], **Clarke O**[*], Zhang K[*], Gennis R[*]. Cryo-EM structures of *Escherichia coli* cytochrome bo_3 reveal bound phospholipids and

ubiquinone-8 in a dynamic substrate binding site. Proc Natl Acad Sci U S A. 2021 Aug 24;118(34). doi: 10.1073/pnas.2106750118.

Complete list of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1Nur6sw6gnmkw/bibliography/public/

BIOGRAPHICAL SKETCH

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

NAME: Huan Li

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

POSITION TITLE: Associate Research Scientist

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
Henan Agricultural University, Zhengzhou, China	B.S.	7/2011	Veterinary medicine
Henan Agricultural University, Zhengzhou, China	M.S.	7/2014	Preventive veterinary medicine
Kunming Institute of Zoology, Kunming, China	Ph.D.	1/2018	Ion channel
Columbia University, New York, NY, US	Ph.D.	8/2021	Cryo-EM, Ion Channel
Columbia university Irving medical center	Ph.D.	Present	Cryo-EM, RyR1

A. Personal Statement

I have been working on ion channels as a graduate student at the Kunming Institute of Zoology since 2014. My Ph.D. thesis was on the screening of natural molecules inspired by Chinese traditional medicine that target ion channels and studying the antiarrhythmic mechanism of compounds that interact with ion channels. I obtained extensive training and experience in electrophysiology, including two electrode voltage clamp(TEVC), patch clamp recording (whole-cell patch, inside-out patch, outside-out patch) and data analysis.

I began to study cryo-electron microscopy (cryo-EM) since I joined Dr. Yang's lab as a postdoc in the department of Biological Sciences at Columbia University. I worked on the structural and functional study of TRPML channels and eukaryotic cyclic nucleotide-gated (CNG) channels, namely ceTAX-4 and hCNGA3/CNGB3 complex. We solved the cryo-EM structures of TAX-4 reconstituted in lipid nanodiscs in cGMP-unbound closed state and cGMP-bound open state at 2.8 Å and 2.7 Å resolutions, respectively. I performed the electrophysiology and biochemistry experiments to verify the ion permeability for gate-related mutations of TAX-4 channel. Besides, we also structurally and functionally characterized a disease-causing mutation of CNG channel R410W. For TRPML channels, my work was focusing on solving the cryo-EM structures of TRPML3 in different PIP2-modulated states. I learned and mastered skills/methods in structural biology, including protein expression and purification, cryo-EM single particle analysis, model building and refinement, and structure analysis and presentation. I have successfully solved the cryo-EM structure of full length TRPML3 with PI(3,5)P2 in pH 4.8 at the resolution of 3.76 Å.

After joining Dr. Clarke's lab as an associate research scientist in the department of Anesthesiology, I have been mainly focused on structure, function, regulation and disease mechanisms of ryanodine receptor 1 (RyR1) using different kinds of approaches including cryo-EM, biochemistry, cell biology and electrophysiology. Dr. Clarke has solved the cryo-EM structure of RyR1 in the closed and open state. Currently, I'm working on solving

the structure of RyR1 in complex with different ligands/small molecules. So far, I have obtained the structures of RyR1 and CLIC2 complex, and discovered the binding site of CLIC2.

1. Li, L.*, **Li, H.***, Peng, X R, Hou, B., Yu, M Y., Dong, J R., Li, X N., Zhou, L., Yang, J., and Qiu, M. (2016). (±)-Ganoapplanin, a Pair of Meroterpenoid Dimers from Ganoderma applanatum. Org. Lett. 18, 6078-6081. (*equal contribution)
2. Zhou, X.[*], Li, M.[*], Su, D.[*], Jia, Q., **Li, H.**, Li, X.[#] & Yang, J.[#] (2017). Cryo-EM structures of the human endolysosomal TRPML3 channel in three distinct states. *Nat. Struc. Mol. Biol.* 24, 1146-1154.
3. 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.
4. Li, M-H[*]., Zhang, W,K.[*], Benvin, N[*]., Zhou, X., Su, D., **Li,H.**, Wang, S., Michailidis, I.E., Tong, L., Li, X., and Yang, J (2017). Structural basis of Ca^{2+}/pH dual regulation of the endolysosomal Ca^{2+} channel TRPML1. *Nat. Struc. Mol. Biol.* 24, 205–213.
5. Zheng, X.*, Fu, Z.*, Su, D.*, Zhang, Y.*, Li, M., Pan, Y., ... & Yang, J. (2020). Mechanism of ligand activation of a eukaryotic cyclic nucleotide− gated channel. *Nat. Struc. Mol. Biol*. 27(7), 625-634.
6. Zheng, X.*, **Li, H**.*, Hu, Z.*, Su, D., & Yang, J. (2022). Structural and functional characterization of an achromatopsia-associated mutation in a phototransduction channel. *Communi bio*, 5(1), 1-13. (*equal contribution)
7. Zheng, X., Hu, Z., **Li, H**., & Yang, J. (2022). Structure of the human cone photoreceptor cyclic nucleotide-gated channel. *Nat. Struc. Mol. Biol*. 29(1), 40-46.

B. Positions and Honors

Positions and Employment

1/2018 – 7/2021, Postdoctoral fellow, Columbia University, Dept. of Biological Sciences
8/2021 – present, Associated research scientist, Columbia University Irving medical center, Dept. of Anesthesiology.

C. Contribution to Science

1. During my Ph.D. thesis, I worked on the screening of natural molecules inspired by Chinese traditional medicine that target ion channels and studying the antiarrhythmic mechanism of compounds that interact with ion channels (Li. et. al., 2016). I identified one compound, namely the natural product AA-1, that showed high inhibition toward multiple types of ion channels. Furthermore, I studied the antiarrhythmic mechanism of AA-1 on the molecular, cellular and animal levels. In the heterogeneous expression system, AA-1 inhibited a variety of cardiac ion channels, including voltage-gated sodium, potassium and calcium channels. AA-1 shortened the action potential duration in guinea-pig ventricular myocytes and changed the waveform of the action potentials. In a rabbit arrhythmia model induced by aconitine, which causes ventricular premature beating, ventricular flutter, atrioventricular block and other malignant arrhythmia, various doses of AA-1 effectively reversed the arrhythmia caused by aconitine. Our results suggested that AA-1 showed high antiarrhythmic effect by synergistically inhibiting multiple ion channels and provided a starting point for the development of a new antiarrhythmic medicine.

    a. Li, L.*, **Li, H.***, Peng, X R, Hou, B., Yu, M Y., Dong, J R., Li, X N., Zhou, L., Yang, J., and Qiu, M. (2016). (±)-Ganoapplanin, a Pair of Meroterpenoid Dimers from Ganoderma applanatum. Org. Lett. 18, 6078-6081. (*equal contribution)
    b. Li, H., Yang, J. Discovery and characterization of an anti-arrhythmia natural product targeting multiple types of ion channels (In preparation).

2. During my postdoctoral research in Yang lab at Columbia University, I was working on structural and functional study of TRPML channels and eukaryotic cyclic nucleotide-gated (CNG) channels and contributed to the papers on TRPML1, TRPML3 and CNG channels. TRPML1 channel primarily located in the membrane of intracellular organelles, especially late endosomes and lysosomes, and played crucial roles in the endocytic pathway. TRPML1 mutations may cause mucolipidosis type IV (ML IV), a severe lysosomal storage disorder. The I-II loop of TRPML1 channel faces to the lysosome lumen and is critical for TRPML1 channel function. Our studies demonstrated that Ca^{2+} and H^{+} ions interact with the luminal pore and exerted physiologically important regulation (Li. et. al., 2017). TRPML3 channels were also mainly localized in endosomes and lysosomes. We determined the cryo-EM structures of full-length human TRPML3 in the apo, ML-SA1-bound open, and low pH-inhibited states. Those structures, combined with mutagenesis and electrophysiological studies, revealed mechanisms of agonist-induced gating, low pH-induced acute inhibition and long-lasting inhibition for TRPML3 (Zhou. et. al., 2017). CNG channels are essential for vision and olfaction. We determined a 3.5 Å-resolution cGMP-bound open-state cryo-EM structure of a full-length eukaryotic CNG channel formed by TAX-4, a CNGA subunit from *C. elegans* (Li. et. al., 2017). We later solved the cryo-EM structures of TAX-4 reconstituted in lipid nano discs in a cGMP-unbound closed state and a cGMP-bound open state at 2.8 Å and 2.7 Å resolutions, respectively (Zheng et al., 2020). Our structure is the first high-resolution full-length structure of CNG channels and provides insights into CNG channel ion permeation, gating and channelopathy. In addition, we structurally and functionally characterized an achromatopsia causing mutation of CNG channel R410W on CNGA3 (Zheng et al., 2022). We made the equivalent mutation on TAX-4 and solved the structure of the mutant. We also performed functional experiments such as calcium signaling and single channel recording using native human CNGA3_R410W/CNGB3 complex. Eventually, we concluded that, in contrary to common belief that R410W is a loss-of-function mutation, it is actually a gain-of-function mutation and causes CNG channel to spontaneously open and eventually induces cell death. This work calls for a reevaluation of other reported loss-of-function disease causing mutations of CNG channels and has implications for mutation-specific treatment of retinopathy.

   a. Zhou, X.[*], Li, M.[*], Su, D.[*], Jia, Q., **Li, H.**, Li, X.[#] & Yang, J.[#] (2017). Cryo-EM structures of the human endolysosomal TRPML3 channel in three distinct states. *Nat. Struc. Mol. Biol.* 24, 1146-1154.
   b. 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.
   c. Li, M-H[*]., Zhang, W,K.[*], Benvin, N[*]., Zhou, X., Su, D., **Li,H.**, Wang, S., Michailidis, I.E., Tong, L., Li, X., and Yang, J (2017). Structural basis of Ca^{2+}/pH dual regulation of the endolysosomal Ca^{2+} channel TRPML1. *Nat. Struc. Mol. Biol.* 24, 205–213.
   d. Zheng, X.*, Fu, Z.*, Su, D.*, Zhang, Y.*, Li, M., Li, H., Li, S., Grassucci, RA., Li, X., Li, G., Frank, J., and Yang, J. (2020). Mechanism of ligand activation of a eukaryotic cyclic nucleotide−gated channel. Nat Struct Mol Biol. 27, 625–634.
   e. Zheng, X.*, Fu, Z.*, Su, D.*, Zhang, Y.*, Li, M., Pan, Y., ... & Yang, J. (2020). Mechanism of ligand activation of a eukaryotic cyclic nucleotide− gated channel. *Nat. Struc. Mol. Biol*. 27(7), 625-634.
   f. Zheng, X.*, **Li, H**.*, Hu, Z.*, Su, D., & Yang, J. (2022). Structural and functional characterization of an achromatopsia-associated mutation in a phototransduction channel. *Communi bio*, 5(1), 1-13. (*equal contribution)
   g. Zheng, X., Hu, Z., **Li, H**., & Yang, J. (2022). Structure of the human cone photoreceptor cyclic nucleotide-gated channel. *Nat. Struc. Mol. Biol*. 29(1), 40-46.

D. Research Support

None