OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

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: Alec Kittredge

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

POSITION TITLE: Graduate Student Research Assistant

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Rochester	BS	08/2013	05/2017	Biology: Neuroscience
Columbia University	PhD	08/2019	TBD	Pharmacology and Molecular Therapeutics

A. Personal Statement

My long-term research interests lie in uncovering the relationships between structure and function of ion channels, and furthermore how these relationships manifest in human diseases. Specifically, my goal is to use cutting-edge technologies like single-particle cryogenic electron microscopy (CryoEM) and whole-cell patch clamp to uncover these relationships. My academic coursework and training have provided me with a solid foundation to pursue these goals, reaching back to my high school career. In high school, I attended a charter-magnet public school that offered courses not usually found in other high schools, such as linear algebra, differential equations, a course on nuclear physics, etc. while balancing an after-school job. While enrolled at the University of Rochester, I had originally planned to go to medical school. However, interested in the publications I read in my courses, the idea of conducting my own research seemed interesting and I subsequently joined the lab of Dr. Greg Tall in the University of Rochester Medical Center's Department of Pharmacology and Physiology. The lab focuses on elucidating the influence of enzymes that interact with G-proteins apart from G-protein coupled receptors. Following Dr. Tall's departure from the University of Rochester, I joined the lab of Dr. Tingting Yang as an undergraduate assistant. The lab focuses on the structure-function relationship of bestrophin proteins, which are calcium-activated chloride channels. Excitingly, my work resulted in my first co-authorship paper in 2017. Following my graduation from the University of Rochester, my interests in the field expanded, and I began to identify the structural impact of different mutations via X-ray crystallography. I simultaneously generated pluripotent stem cell (PSC)-derived retinal pigment epithelium (PSC-RPE) to illuminate the influence of human Best1 (hBest1) mutations from a functional viewpoint. These works culminated in numerous publications. Between my time as an undergraduate assistant to now, I have published ten publications on the topic, including a co-first-author paper and three first-author methods articles, in addition to two other works current under review. Now as a PhD candidate at Columbia University in the same lab, I look forward to continuing and expanding my work on deciphering the mechanisms of Best1 gating mechanisms from a structural perspective. If awarded, this fellowship will support the continuation of my training in the field of membrane protein structure-function relationships and expand this research into clinically viable therapies.

B. Positions and Honors

Positions and Employment

2016 - 2016 Teaching Assistant, University of Rochester Department of Chemistry
2015 - 2016 Lab Assistant, University of Rochester Department of Pharmacology and Physiology

2016 - 2017 Lab Assistant, University of Rochester Department of Pharmacology and Physiology
2017 - 2019 Lab Technician, University of Rochester Department of Pharmacology and Physiology
2019 - Present Graduate Research Assistant, Columbia University Department of Pharmacology and Molecular Therapeutics

Professional Meetings, Posters, and Presentations

2018 Attended and presented research poster at Association for Research in Vision and Ophthalmology (ARVO) 2018 annual meeting
2018 Attended and presented research poster at University of Rochester 2018 Genetics Day poster symposium
2019 Attended and presented research poster at Biophysical Society 2019 annual meeting
2020 Attended American Crystallographic Association 2020 annual meeting
2022 Attended and presented research poster at Biophysical Society 2022 annual meeting

Honors

2013 - 2017 Dean's List (7/8 semesters), University of Rochester

Other

2014 - 2017 Member (2014-2015), Secretary (2015-2016), Business Manager (2016-2017), University of Rochester Student Programming Board
2020 - Present Writer for Columbia scientific blog group PhDish

C. Contributions to Science

I. **Undergraduate Research at University of Rochester:** Shortly before I was hired as an undergraduate assistant in her lab, Dr. Yang had solved the structure of *Klebsiella pneumoniae* Best (KpBest), a bacterial homolog of the hBest1 calcium-activated chloride channel. Intent on deciphering the influence of patient-derived Best1 mutations, I assisted in this goal by maintaining our HEK293 cell lines, induced pluripotent stem- retinal pigment epithelium (iPSC-RPE) cells, and generated hBest1 mutant constructs by site-directed mutagenesis. This work resulted in identifying the patient-derived autosomal recessive mutations I201T and P274R as loss-of-function and correlated their structural changes to the functional disruptions. The I201T construct and the cells I maintained were used in the below Nature Communications paper, which identified this residue as an ATP-binding site. Also, the iPSC-RPE cells I maintained were used to verify these as an *in vivo* model of *BEST1* mutations.

i. Li Y, Zhang Y, Xu Y, **Kittredge A**, Ward N, Chen S, Tsang SH, Yang T. "Patient-specific mutations impair BESTROPHIN1's essential role in mediating Ca-dependent Cl currents in human RPE." eLife. 2017 Oct 24; 6Epub 2017 Oct 24.
ii. Zhang Y, **Kittredge A,** Ward N, Ji C, Chen S, Yang T. "ATP activates bestrophin ion channels through direct interaction." Nature communications. 2018 Aug 7; 9(1):3126. Epub 2018 Aug 07.

II. **Research Technician at University of Rochester:** Following my graduation from the University of Rochester in 2017, I stayed with the lab and was hired as a full-time technician, where I continued my research into the structure and function of bestrophin proteins. In addition to generating constructs, I learned the protein expression, purification, and X-ray crystallography protocols to crystallize KpBest. Continuing to investigate the structure and function of bestrophin proteins, I used KpBest to decipher the structural influence of numerous patient-derived mutations. For example, I identified a network of key residues, including Y236 and W287, that contribute to the proposed neck and aperture gating mechanism of hBest1. I simultaneously utilized a line of human pluripotent stem cells (hPSCs) with a Doxycycline-inducible CRISPR/Cas9 cassette to generate retinal pigment epithelium (hPSC-RPE) cells with RNA-targeted mutations. These cells were used to determine the expression requirements and rescue strategies for hBest1 mutants such as Y236C and I205T. Cells generated during this time were also used to identify hBest1 as the *bona fide* CaCC in RPE cells (Zhao et al., 2021). During this time, I also wrote two methods articles on the protocols we used to generate this data.

i. **Kittredge A**, Ji C, Zhang Y, Yang T. "Differentiation, Maintenance, and Analysis of Human Retinal Pigment Epithelium Cells: A Disease-in-a-dish Model for BEST1 Mutations." Journal of visualized experiments: JoVE. 2018 Aug 24; (138) Epub 2018 Aug 2

ii. **Kittredge A,** Ward N, Hopiavuori A, Zhang Y, Yang T. "Expression and Purification of Mammalian Bestrophin Ion Channels." Journal of visualized experiments: JoVE. 2018 Aug 2; (138)Epub 2018 Aug 02.

iii. Ji, C.,[#] **Kittredge, A**.,[#] Hopiavuori, A., Ward, N., Chen, S., Fukuda, Y., Zhang, Y., Yang, T. (2019). Dual Ca2+ -dependent gates in human Bestrophin1 underlie disease-causing mechanisms of gain-of-function mutations. *Communications Biology, 2*(1). doi:10.1038/s42003-019-0433-3
[#]These authors contributed equally.

iv. Ji, C., Li, Y., **Kittredge, A**. *et al.* Investigation and Restoration of BEST1 Activity in Patient-derived RPEs with Dominant Mutations. *Sci Rep* 9, 19026 (2019). https://doi.org/10.1038/s41598-019-54892-7

III. Graduate Research at Columbia University: I enrolled in the Pharmacology and Molecular Therapeutics PhD program at Columbia University knowing that I wanted to continue working on ion channels. Here, I would re-join the Yang Lab and continue researching bestrophin proteins. My first two projects since re-joining the lab focused on solving the structure of the hBest1 and hBest2 proteins by CryoEM and identifying physiologically relevant binding partners of bestrophin channels. The structures of the hBest1 and hBest2 were recently solved by our group to 1.8-2.3 Å each, and I identified a physiologically relevant interaction between hBest2 and a cytosolic enzyme. With the structures of the human bestrophin proteins solved, I will continue to elucidate the mechanisms of Ca^{2+}-dependent gating and work towards identifying an ATP-bound bestrophin structure to determine the cooperative mechanism between the two signaling molecules on bestrophins.

i. Zhao, Q., Kong, Y., **Kittredge A**., Li, Y., Shen, Y., Zhang, Y., Tsang, S.H., Yang, T. (2021). Distinct epigenetic requirements and rescue strategies for *BEST1* loss- and gain-of-function mutations. *eLife.* doi: 10.7554/eLife.67622

ii. Owji, A., Wang, J., **Kittredge, A**., Clark, Z., Zhang, Y., Hendrickson, W., Yang, Y. (2022). Structures and gating mechanisms of human bestrophin anion channels. *Under Review at Nature Communications.*

iii. Owji, A., **Kittredge, A**., Zhang, Y., Yang, T. (2021). Structure and Function of the Bestrophin Family of Calcium-activated Chloride Channels. *Channels, 15,* 604-623. doi: 10.1080/19336950.2021.1981625

iv. Owji, A. P., Wang, J., **Kittredge, A**., Clark, Z., Zhang, Y., Hendrickson, W. A., & Yang, T. (2022). Structures and gating mechanisms of human bestrophin anion channels. Nature communications, 13(1), 3836. https://doi.org/10.1038/s41467-022-31437-7

Complete List of Published Work:
https://pubmed.ncbi.nlm.nih.gov/?term=Alec+Kittredge

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

YEAR	COURSE TITLE	GRADE
UNIVERSITY OF ROCHESTER		
2013	Biology Perspectives I	A
2013	Chemical Concepts, Systems, and Practices I	A-
2013	Linear Algebra with Differential Equations	A
2013	Introduction to Biomedical Engineering	A-
2014	Chemical Concepts, Systems, and Practices II	A
2014	Multidimensional Calculus	A
2014	Mechanics	A-
2014	Hollywood Genre Film	A-
2014	Organic Chemistry I	A-
2014	Organic Chemistry I: Lab Lecture	A-
2014	Basic Neurobiology	B
2014	Basic Neurobiology Lab	A
2014	Introduction to the U.S. Health System	B+
2014	Electricity & Magnetism, Self-Paced	B+
2015	Principles of Biology II	A
2015	Introductory Biology Lab	A
2015	Organic Chemistry II	B+

2015	Organic Chemistry II: Lab Lecture	A
2015	Health, Medicine, and Social Reform	B+
2015	Introduction to Public Health	A
2015	Applied Statistics – Biology, Physics, and Science	A
2015	Principles of Genetics	A
2015	Principles of Genetics Lab	A
2015	Introduction to Biochemistry	A
2015	Beginning American Sign Language I	A
2015	Chemistry 203 Workshop – Leadership - A	A
2015	Neuropsychology	B
2015	Neurochemistry Foundations of Behavior	B
2015	Peer Health Advocacy	A
2016	Beginning American Sign Language II	A
2016	Transition to Higher Mathematics	A
2016	Lab in Neurobiology	B
2016	Neuroethology	A
2016	Mammalian Physiology	A
2016	Mammalian Physiology - Lab	A
2016	Linear Algebra	A
2016	Intermediate American Sign Language I	A
2016	Developmental Biology	A-
2016	Introduction to Financial Mathematics	B+
2016	Senior Seminar in Neuroscience	A-
2016	Web Page Design & Development	A
2017	The Chemistry of Poisons	A
2017	International & Global Health	A
2017	Public Health Anthropology	A
2017	Criminal Procedure & Constitutional Principles	S

COLUMBIA UNIVERSITY

2019	Biochemistry/Molecular/Cell Biology	B
2019	Principles of Systems Pharmacology	A
2019	Advances in Pharmacology	P
2019	Pharmacology Techniques I	A
2020	Biochemistry/Cell/Molecular Biology	P
2020	Advances in Pharmacology	P
2020	Pharmacology Techniques I	P
2020	Molecular Pharmacology: Membrane - Nucleus	P
2020	Mechanisms in Human Disease	A
2020	Research in Pharmacology	A
2021	Responsible Conduct of Research and Related Policy Issues	P
2021	Structure and Function of Membrane Channels	B+
2021	Advances in Pharmacology	A+
2021	Statistics for Basic Sciences	A
2021	Research in Pharmacology	A

At the University of Rochester, Criminal Procedure and Constitutional Principles was graded as S (Satisfactory) or F (fail). A grade of C or better is considered satisfactory.

P (pass) or F (fail) grades were given to all students following the impacts of the coronavirus pandemic in the spring of 2020. Advances in Pharmacology is given as pass/fail for the first year (2019-2020) and is a letter grade for the second year (2020-2021). The Responsible Conduct of Research and Related Policy Issues is always given a pass/fail grade. A grade of a C plus or better is considered a pass.

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: Aaron P. Owji

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

POSITION TITLE: Postdoctoral Researcher

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 Central Florida, Orlando, FL	BS	08/2012	Biotechnology & Molecular Biology
University of Central Florida, Orlando, FL	MS	06/2015	Biotechnology (w/ Thesis)
Columbia University, Graduate School of the Arts and Sciences, New York, NY	MA	08/2016	Pharmacology & Molecular Signaling
Columbia University, Graduate School of the Arts and Sciences, New York, NY	MPhil	08/2017	Pharmacology & Molecular Signaling
Columbia University, Graduate School of the Arts and Sciences, New York, NY	PhD	02/2022	Pharmacology & Molecular Signaling

A. Personal Statement

My long-term research interest lies in elucidating the structures of integral membrane proteins related to human health and disease. Specifically, I seek to utilize single-particle cryogenic electron microscopy (cryoEM) and X- ray crystallography to determine the structure and functional relationship of biomedically-relevant protein targets. My academic coursework and training have provided me with an excellent understanding of molecular biology, physiology, biochemistry, and, more recently, a variety of structural techniques. As an undergraduate at the University of Central Florida, I sought my initial training in biomedical research in the lab of Dr. Steven N. Ebert, where I developed a strong interest in cardiac physiology. Upon graduating with my Bachelor's degree, I began my Master's coursework and continued working with Dr. Ebert for my Master's thesis. My research aim was to determine how a specific population of progenitor cells, which express the biosynthetic enzyme for adrenaline, contribute to heart development and adult cardiac function. I presented work from my Master's thesis at two major conferences with the American Heart Association and I was also selected for 1st Place Master's Presentation at the University of Central Florida 9th Annual Graduate Research Symposium. This initial work in cardiac physiology, which entailed in-depth mouse echocardiography, led to a fascination with ion channel function and my ultimate pursuit of a PhD in the Pharmacology and Molecular Signaling program at Columbia University. By the time I joined Columbia, I had a keen interest in electrophysiology and this interest grew as I learned more about the structural mechanisms underlying ion channel function. For my doctoral dissertation, I am generating a structural model to explain calcium-dependent activity of mammalian bestrophins. Other projects I am developing include elucidation of the molecular mechanisms underlying activity of the Tweety homolog family of volume regulated anion channels, as well as the structural basis of organic anion transport by an OATP transporter. The common theme of these projects is that they are membrane proteins of biomedical significance that require thorough biochemical optimization for successful structural analysis. Our access to NCCAT microscope resources will further the development of these membrane protein projects and will directly contribute to my development as a scientist in training.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2011-2012	Undergraduate Research Volunteer, 1 year, University of Central Florida
2012-2014	Graduate Teaching Assistant in Microbiology, University of Central Florida
2014-2015	Graduate Research Assistant, University of Central Florida
2013-2015	Event Planning Committee Member, UCF Biomedical Sciences Graduate Student Association
2015-2022	Graduate Research Assistant, Columbia University
2015-2016	Graduate Student Organization Social Committee, Columbia University
2017-2019	Mentor for High School Students in the Minds Matter Science Matters Research Internship
2022-present	Postdoctoral Researcher, Columbia University

Academic and Professional Awards

2008-2012	Selected as Florida Bright Futures Medallion Scholar, which paid 75% of tuition at all Florida public universities for four years
2009-2012	Dean's List, University of Central Florida, 6 semesters
2011-2012	Active Member of Delta Epsilon Iota UCF Chapter, Academic Honor Society
2014	Selected for Kalyani Parthasarathy Award for 1st Place M.S. Presentation at the UCF 9th annual Graduate Research Symposium, which included a cash prize
2017-2018	Selected for the Training Program in Molecular Biophysics, Training Grant T32 5T32GM008281-30, NIGM
2018	Selected as Fisher Award Recipient based on research progress (Columbia Internal Award - covered registration costs at the COMPPÅ Symposium on Membrane Protein Production and Analysis)
2019-2022	Ruth L. Kirschstein National Research Service Award (NRSA / F31)
2022	1st Place in Student Research Achievement Award (SRAA) Poster Competition at the 66th Biophysical Society Annual Meeting, CryoEM subgroup

Memberships in Professional Societies

2013-2015	American Heart Association, Student/Trainee Member
2015-2016	NYAS, Student Member
2020-2021	American Crystallographic Association, Student Member
2021-2022	Biophysical Society, Student Member

Professional Meetings, Posters, and Presentations

2014	Selected for Poster Presentation, "Genetically-programmed suicide of adrenergic cells in mice produces left ventricular dysfunction as revealed by high-resolution echocardiography." Abstract #17028. At the American Heart Association Scientific Sessions in Chicago, IL.
2015	Selected for Poster Presentation, "Selective destruction of adrenergic cells in mice leads to severe left-ventricular dysfunction at rest with apparent stress-induced recovery." Abstract #197. At the American Heart Association Basic Cardiovascular Sciences (BCVS) Scientific Sessions in New Orleans, LA.
2016	Attended New York Structural Biology Discussion Group Summer Meeting
2017	Attended New York Structural Biology Discussion Group Winter Meeting
2017	Attended New York Structural Biology Discussion Group Summer Meeting
2017	Attended Center on Membrane Protein Production and Analysis (COMPPÅ) Annual Meeting
2018	Attended New York Structural Biology Discussion Group Winter Meeting
2018	Attended New York Structural Biology Discussion Group Summer Meeting
2018	Attended Center on Membrane Protein Production and Analysis (COMPPÅ) Symposium on Membrane Protein Production and Analysis, Fisher Award Recipient
2020	Poster Presentation (Canceled due to COVID-19) at Understanding Biology Through Structure 2020.

2021	Selected for Poster Presentation, “Structural and Functional Characterization of the Bestrophin-2 Anion Channel.” At the Biophysical Society 2021 Annual Meeting. (Virtual).
2022	Owji AP. Structural and functional characterization of the Bestrophin2 anion channel. Presentation L1959-Pos. Presented at BPS2021 65th Biophysical Society Annual Meeting (Virtual); Feb. 26, 2021.
2022	CryoEM analysis of gating dynamics in mammalian bestrophins. Presented at BPS2022 66th Biophysical Society Annual Meeting; Feb. 22, 2022.

C. Contributions to Science

C.1 Undergraduate Research at the University of Central Florida. I spent one year volunteering in the lab of Dr. Steven Ebert at the University of Central Florida. During this time, I learned basic lab techniques used to study heart development. This was my first exposure to hands-on biomedical research and it led to my pursuit of a Master’s of Science with a thesis.

C.2 Master of Science Thesis at The University of Central Florida. I worked in Dr. Ebert’s lab for one year prior to beginning my thesis work in the Biotechnology MS program. I found the field of cardiovascular development exciting and led a study to identify the role of a specific cardiomyocyte progenitor cells in heart development. My work focused on the role of progenitor cells that express phenylethanolamine-N-methyltransferase (Pnmt), the biosynthetic enzyme for adrenaline, and their contribution to working myocardium in the adult. I received an award for 1st place Master’s Presentation for my oral presentation of this work at the UCF 9th Annual Graduate Research Symposium in 2014. My completion of this program required formation of a thesis committee, an oral thesis defense, and a written thesis submission. I was also a Graduate Teaching Assistant for these three years and received a full tuition waver and a yearly stipend.

1. **Owji, AP**, Genetically-programmed suicide of adrenergic cells in the mouse leads to severe left ventricular dysfunction, impaired weight gain, and symptoms of neurological dysfunction. (**2015**). *Electronic Theses and Dissertations*. 1492. https://stars.library.ucf.edu/etd/1492
2. **Owji AP**, Varudkar N, Ebert SN. Therapeutic potential of Pnmt+ primer cells for neuro/myocardial regeneration. American Journal of Stem Cells. **2013**;2(3):137-54. Epub 2014/01/08. PMID:24396707
3. **Owji AP**, Baker CN, Jacob JL, Tumuluri L, Ebert SN. Genetically-programmed suicide of adrenergic cells in the mouse leads to severe left ventricular dysfunction, impaired weight gain, and neurological dysfunction. (Manuscript in preparation)
4. Baker CN, Katsandris R, **Owji AP**, Goldblatt G, Van C, and Ebert SN. Echocardiographic and Histological Analysis of Left Ventricular Function in Stress-Challenged Aged Mice: Effects of Gender and Menopause. (Manuscript in preparation)

C3. Graduate Research at Columbia University
My ongoing predoctoral research is focused on understanding the molecular mechanisms of calcium-dependent activation and inactivation in mammalian bestrophin channels. Specifically, I use cryoEM to study how this channel responds to activating and inactivating levels of calcium, as well as the mechanism of potentiation by ATP. Bestrophins are a family of Ca^{2+}-activated Cl^{-} channels expressed in a variety of human tissues. The Best2 isoform is localized to the basolateral plasma membrane of nonpigmented ciliary epithelial cells of the nonpigmented epithelium of the ciliary body and is required for the maintenance of intraocular pressure. I have recently used cryoEM to solve the first structure of a mammalian bestrophin channel, which is also the first Best2 structure. These structures, coupled with functional experiments, reveal regions of the channel responsible for gating and selectivity and have distinct differences from the Best1 channel. Ongoing areas of investigation on this project include structural analysis of human bestrophins and mechanisms of general chloride channel inhibitors.

1. **Owji AP**, Zhao Q, Ji C, Kittredge A, Hopiavuori A, Fu Z, Ward N, Clarke OB, Shen Y, Zhang Y, Hendrickson WA, Yang T. Structural and functional characterization of the bestrophin-2 anion channel. Nat Struct Mol Biol. 2020 Apr;27(4):382-391. doi: 10.1038/s41594-020-0402-z. Epub 2020 Apr 6. PMID: 32251414; PMCID: PMC7150642.

2. **Owji AP**, Wang J, Kittredge A, Clark Z, Zhang Y, Hendrickson WA, Yang T. Structures and gating mechanisms of human bestrophin anion channels. Nat Commun. 2022 Jul 4;13(1):3836. doi: 10.1038/s41467-022-31437-7. PMID: 35789156; PMCID: PMC9253114.
3. **Owji AP**, Yu K, Kittredge A, Wang J, Zhang Y, Yang T. Bestrophin-2 and glutamine synthetase form a complex for glutamate release. Nature. 2022 Nov;611(7934):180-187. doi: 10.1038/s41586-022-05373-x. Epub 2022 Oct 26. PMID: 36289327.

C4. Postdoctoral research at Columbia University

My postdoctoral research is focused on understanding potential mechanisms by which human bestrophin channels may be modulated for pharmacological or research purposes. I have used cryoEM to identify compounds capable of activating human bestrophins and will continue to optimize compounds to increase binding affinity and potency.

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: Tingting Yang

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

POSITION TITLE: Associate Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Fudan University, Shanghai, China	B.S.	07/2001	Biological sciences
Fudan University, Shanghai, China	M.S.	07/2004	Microbiology
Johns Hopkins University, Baltimore, MD	M.S.E.	05/2008	Applied Math and Statistics
Johns Hopkins University, Baltimore, MD	Ph.D.	05/2010	Ion channel function (Colecraft lab)
Columbia University, New York, NY	Postdoc	05/2010-08/2012*	Ion channel function (Colecraft lab)
Columbia University, New York, NY	Postdoc	09/2012-12/2015	Ion channel structure (Hendrickson lab)

**09/10-08/11: left science for family reasons. Colecraft lab moved from Hopkins to Columbia in 2007.*

A. Personal Statement

Employing multidisciplinary approaches including cryoEM, crystallography, electrophysiological recording, CRISPR/Cas9, gene therapy and stem cell reprogramming/differentiation, my lab studies the structure, function and regulation of bestrophins, a family of Ca^{2+}-activated anion channels with important (patho)physiological implications in human eyes. Bestrophin-1 (Best1) is predominantly expressed in the retinal pigment epithelium (RPE), and mutations in the human *BEST1* gene result in a spectrum of retinal degenerative disorders known as bestrophinopathies. Bestrophin-2 (Best2) is located in non-pigmented epithelium (NPE) of the ciliary body, participating in the regulation of intra-ocular pressure (IOP). My previous works include solving the first Best1 and Best2 structures, characterizing their functions and physiological roles, elucidating disease-causing mechanisms of *BEST1* patient-derived mutations, and developing gene therapy for bestrophinopathies.

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

R35 GM149252 (Yang, PI)
NIH/NIGMS 04/01/2023 – 03/30/2028
Interacting partners of bestrophin channels
This project aims to study the bestrophin-associated interactomes in the eye and their (patho)physiological roles.

R01 GM127652 (Yang, PI) 05/01/2018 – 03/30/2024
NIH/NIGMS

Mechanistic characterization of calcium-activated chloride channels in retinal pigment epithelium
This project aims to define the physiological contributions of BEST1, TMEM16A and TMEM16B channels to the Ca^{2+}-dependent Cl^- fluxes in retinal pigment epithelium.

R24 EY028758 (Yang, I) 06/01/2020 – 05/31/2025
NIH/NEI
Therapeutic gene editing and multimodal imaging in juvenile macular degeneration
This project aims to establish CRISPR/Cas9-mediated gene correction therapy for juvenile macular degeneration.

Irma T. Hirschl Research Award (Yang, PI) 01/01/2021 – 12/31/2025
Irma T. Hirschl Trust
Structural and functional investigations of BEST1 patient-derived mutations
This project aims to investigate the epigenetics and patient-specific effects that cause distinct clinical phenotypes in different individuals with the same *BEST1* genotype.

Career Advancement Award (Yang, PI) 01/01/2023 – 12/31/2024
Research to Prevent Blindness
Best1-mediated anion transport in retinal pigment epithelium
This project aims to elucidate the molecular mechanisms underlying how the Best1 channel conducts physiological anions in the eye.

Collaboration with Opus Genetics (Yang, PI) 01/01/2023 – 12/31/2023
Electrophysiological assessment of Best1 mutations
This project aims to systematically evaluate how patient-derived dominant mutations impact Best1-mediated Ca^{2+}-dependent Cl^- currents in HEK293 cells.

K99/R00 EY025290 (Yang, PI) 05/01/2015 – 12/31/2019
NIH/NEI
Structure-function analysis of bestrophins
This project was focused on elucidating the basic structure and function of Best1 using a bacterial homolog as a model.

Citations:

1. Owji AP, Yu K, Kittredge A, Wang J, Zhang Y, **Yang T**. Bestrophin-2 and glutamine synthetase form a complex for glutamate release. *Nature*, 2022; 611(7934):180-187
2. Owji AP, Wang J, Kittredge A, Clark Z, Zhang Y, Hendrickson WA, **Yang T**. Structures and gating mechanisms of human bestrophin anion channels. *Nat Commun*, 2022; 13(1):3836
3. Owji AP, Zhao Q, Ji C, Kittredge A, Hopiavuori A, Fu Z, Ward N, Clarke OB, Shen Y, Zhang Y, Hendrickson WA, **Yang T**. Structural and functional characterization of the bestrophin-2 anion channel. *Nat Struct Mol Biol*, 2020; 27(4): 382-391
4. Zhang Y, Kittredge A, Ward N, Ji C, Chen S, **Yang T**. ATP activates bestrophin ion channels through direct interaction. *Nat Commun*, 2018; 9(1): 3126

B. Positions, Scientific Appointments, and Honors

Positions and Employment

Associate Professor, Ophthalmology, Columbia University	2022-present
Assistant Professor, Ophthalmology, Columbia University	2019-2022
Assistant Professor, Pharmacology and Physiology, University of Rochester	2016-2019
Associate Research Scientist, Biochemistry and Molecular Biophysics, Columbia University	2015
Postdoc Research Scientist, Biochemistry and Molecular Biophysics, Columbia University	2012-2015
Postdoc Research Scientist, Physiology and Cellular Biophysics, Columbia University	2010, 2011-2012

Honors

NIH/NIGMS Maximizing Investigators' Research Award (MIRA/R35)	2023

Career Advancement Award, Research to Prevent Blindness	2022
Blavatnik National Faculty Award Nominee in Life Sciences	2022
Schaefer Research Award	2021
Irma T. Hirschl Research Award	2021
Target-of-Opportunity Faculty Recruitment Award, Columbia University	2019
NIH Pathway to Independence Award (K99/R00)	2015
Symposium Award, Society of General Physiologists	2015
Travel Award, Biophysical Society	2012
Phi Beta Kappa National Academic Honor Society	2010
Student Research Achievement Award, Biophysical Society	2009
Student Travel Grant, Biophysical Society	2009
Physiology Retreat Poster Award, 1st Prize, Columbia University	2009

C. Contributions to Science

1. Biophysics of bestrophin channels: We solved the first Best1 and Best2 structures, and found that bestrophins have two Ca^{2+}-dependent channel gates and a C-terminal auto-inhibitory segment that determines paralog specificity among bestrophins.

a. Owji AP, Wang J, Kittredge A, Clark Z, Zhang Y, Hendrickson WA, **Yang T**. Structures and gating mechanisms of human bestrophin anion channels. *Nat Commun*, 2022; 13(1):3836
b. Owji AP, Zhao Q, Ji C, Kittredge A, Hopiavuori A, Fu Z, Ward N, Clarke OB, Shen Y, Zhang Y, Hendrickson WA, **Yang T**. Structural and functional characterization of the bestrophin-2 anion channel. *Nat Struct Mol Biol*, 2020; 27(4): 382-391
c. Ji C, Kittredge A, Hopiavuori A, Ward N, Chen S, Fukuda Y, Zhang Y, **Yang T**. Dual Ca^{2+}-dependent gates in human Bestrophin1 underlie novel disease-causing mechanisms of gain-of-function mutations. *Commun Biol*, 2019; 2:240
d. **Yang T**, Liu Q, Kloss B, Bruni R, Kalathur RC, Guo Y, Kloppmann E, Rost B, Colecraft HM, Hendrickson WA. Structure and selectivity in bestrophin ion channels. *Science*, 2014; 346(6207): 355-9

2. Interacting regulators of bestrophin channels: We identified glutamine synthetase as a paralog-specific binding partner of Best2 to facilitate intracellular glutamate release from NPE cells, and ATP as an evolutionarily conserved interacting activator of bestrophins.

a. Owji AP, Yu K, Kittredge A, Wang J, Zhang Y, **Yang T**. Bestrophin-2 and glutamine synthetase form a complex for glutamate release. *Nature*, 2022; 611(7934):180-187
b. Zhang Y, Kittredge A, Ward N, Ji C, Chen S, **Yang T**. ATP activates bestrophin ion channels through direct interaction. *Nat Commun*, 2018; 9(1): 3126

3. (Patho)physiology of bestrophins: We demonstrated the physiological role of Best1 in mediating Ca^{2+}-dependent Cl^- current in RPE cells, elucidated disease-causing mechanisms of *BEST1* patient-derived mutations, and developed gene therapy for bestrophinopathies.

a. Zhao Q, Kong Y, Kittredge A, Li Y, Shen Y, Zhang Y, Tsang SH, **Yang T**. Distinct expression requirements and rescue strategies for BEST1 loss- and Gain-of-function mutations. *eLife,* 2021; 10: e67622
b. Ji C, Li Y, Kittredge A, Hopiavuori A, Ward N, Yao P, Fukuda Y, Zhang Y, Tsang SH, **Yang T**. Investigation and restoration of BEST1 activity in patient-derived RPEs with dominant mutations. *Sci Rep*, 2019; 9(1): 19026
c. Li Y, Zhang Y, Xu Y, Kittredge A, Ward N, Chen S, Tsang SH, **Yang T**. Patient-specific mutations impair BESTROPHIN1's essential role in mediating Ca^{2+}-dependent Cl^- currents in human RPE. *eLife*, 2017; 6: e29914
d. **Yang T**, Justus S, and Li Y, Tsang SH. BEST1: the best target for gene and cell therapies. *Molecular Therapy*, 2015; 23(12): 1805-9

4. Methods development: We established two "disease-in-a-dish" models. 1) Skin or blood samples are collected from bestrophinopathy patients, reprogrammed into induced pluripotent stem cells (iPSCs) and then

differentiated into RPE (iPSC-RPE) cells, which contain the specific *BEST1* mutations and genetic background from the patients. 2) Desired *BEST1* mutations are generated by CRISPR/Cas9-mediated genome editing in a human pluripotent stem cell (hPSC) line containing a doxycycline-inducible Cas9 cassette (iCas9), followed by differentiation to generate isogenic RPE (hPSC-RPE) cells. These strategies provide a steady source of human originated RPE cells for various experimental analyses, and have been applied to study RPE (patho)physiology and other genes natively expressed in RPE. We also developed expression and purification protocols for mammalian bestrophin proteins.

a. Kittredge A, Zhang Y, **Yang T**. Evaluating BEST1 mutations in hPSC-RPE cells. *Methods Enzymol,* 2021; 654: 365-382
b. Owji AP, Kittredge A, Zhang Y, **Yang T**. Structure and Function of the Bestrophin family of calcium-activated chloride channels. *Channels (Austin)*, 2021; 15(1): 604-623
c. Kittredge A, Ji C, Zhang Y, **Yang T**. Differentiation, maintenance and analysis of human retinal pigment epithelium cells: a disease-in-a-dish model for BEST1 mutations. *J Vis Exp*, 2018; (138): 57791
d. Kittredge A, Ward N, Hopiavuori A, Zhang Y, **Yang T**. Expression and purification of mammalian Bestrophin ion channels. *J Vis Exp*, 2018; (138): 57832

5. Other ion channels: I had worked on the TMEM16 Ca^{2+}-activated anion channels and voltage-activated Ca^{2+} channels in my postdoc and PhD studies. I deciphered their regulatory mechanisms and established a general method for developing novel genetically encoded channel blockers, termed 'channel inactivation induced by membrane-tethering of an associated protein' (ChIMP).

a. **Yang T***, Hendrickson WA*, Colecraft HM*. Preassociated apocalmodulin mediates Ca^{2+}-dependent sensitization of activation and inactivation of TMEM16A/16B Ca^{2+}-gated Cl^- channels. *PNAS*, 2014; 111(51): 18213-8 (*corresponding authors)
b. **Yang T**, He LL, Chen M, Fang K, Colecraft HM. Bio-inspired voltage-dependent calcium channel blockers. *Nat Commun,* 2013; 4: 2540
c. **Yang T**, Xu X, Kernan T, Wu V, Colecraft HM. Rem, a member of the RGK GTPases, inhibits recombinant $Ca_V1.2$ channels using multiple mechanisms that require distinct conformations of the GTPase. *J physiol,* 2010; 588(Pt 10): 1665-1681 (Cover Article)
d. **Yang T**, Suhail Y, Dalton S, Kernan T, Colecraft HM. Genetically encoded molecules for inducibly inactivating Ca_V channels. *Nat Chem Biol,* 2007; 3(12): 795-804

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