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: Simon Vu

eRA COMMONS USER NAME (credential, e.g., agency login): SIMONVU1

POSITION TITLE: 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
University of California, Davis	B.S.	12/2012	Molecular and Cellular Biology, Biochemistry
University of California, Davis	Ph.D.	11/2020	Pharmacology and Toxicology
Van Andel Institute	Postdoc	12/2021	Structural Biology

A. Personal Statement

My research training focused on elucidating the structure-function relationships of TRP channels to understand their activation mechanisms. Through investigating the molecular and biophysical events triggered by various stimuli, this work offered a better understanding of TRP channels under physiological conditions, as well as useful insight into the development and prevention of pain. Thus far, I have employed various approaches ranging from electrophysiological methods (e.g., patch-clamp electrophysiology, kinetic analysis, hidden Markov modeling), computational methods (e.g., Rosetta homology modeling, *de novo* structure prediction, ligand docking), and optical methods (e.g., calcium imaging, patch fluorometry, spectral imaging). My research focused on determining ligand-dependent activation mechanisms of TRP channels as well as their roles in physiology. During my graduate research, I published 10 research articles (with 6 being first-author/co-first author) and received academic awards and funding for my research. For my postdoctoral training, I built upon my previous research elucidating ligand-activating and channel gating mechanisms to further understand the function of ion channels under physiological conditions employing new strategies in structural biology.

In the Kirichok lab at Washington University in St. Louis, my current focus is the molecular physiology of mitochondrial membranes. Using my previous expertise in electrophysiology and structural biology, I am investigating the composition and function of mitochondrial ultrastructure. In addition, I am a scientist at Equator Therapeutics developing anti-obesity drugs that specifically target the thermogenic H^+ currents via the mitochondrial ADP/ATP carrier (AAC).

a. Yang, F.*, **S. Vu***, V. Yarov-Yarovoy, and J. Zheng (2016) Rational design and validation of a vanilloid-sensitive TRPV2 ion channel, *Proceedings of the National Academy of Sciences, USA, E3657–E3666.* *These authors contributed equally to this work. •• *Highlighted by F1000Prime*

b. **Simon Vu**, Vikrant Singh, Heike Wulff, Vladimir Yarov-Yarovoy, Jie Zheng. (2020) New capsaicin analogs as molecular rulers to define the permissive conformation of the mouse TRPV1 ligand binding pocket. *eLife.*

c. C Liu*, R Reese*, **S Vu*** *et al.* (2021) A non-covalent ligand reveals biased agonism of the TRPA1 ion channel. *Neuron*. *These authors contributed equally to this work.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2011 – 2013	Research Assistant, UC Davis, Inoue Lab
2013 – 2015	Junior Specialist, UC Davis, Goldkorn Lab
2015 – 2020	Graduate Student Researcher, UC Davis, Zheng Lab
2020 – 2021	Postdoctoral Researcher, Van Andel Institute, Lü and Du Lab
2021 – 2022	Scientific Developer, Emerald Cloud Lab
2022 – Current	Principal Scientist, Equator Therapeutics
2023 – Current	Senior Scientist, Washington University in St. Louis

Academic and Professional Honors

2015	Poster/Abstract Award – Lung Research Day – UC Davis Internal Medicine: CCRBM
2015	TRDRP Cornelius Hopper Diversity Award Fellowship
2016 – 2019	American Heart Association Predoctoral Fellowship (16PRE29340002)
2019	UC Davis Graduate Student International Travel Award
2019	7th International Ion Channel Conference Poster Award

C. Contributions to Science

1. Ligand-dependent TRPV1 activation mechanism by vanilloid compounds: Ligands use energy derived from chemical binding to shift the equilibrium between closed and open state of many channel types, including polymodal TRP channels. The capsaicin receptor is a great model to understand ligand binding and gating mechanisms of receptors. My research during this period had yielded a number of key findings concerning ligand-induced activation mechanism and dynamic modulation of channel activity. At the time, though the cryo-EM structure of TRPV1 with capsaicin had been resolved, specific residues that interact with this molecule were still not defined. Using a combination of experimental electrophysiology and computational modeling, I was able to determine key TRPV1 residues involved in ligand binding to and residues which were important for gating of the channel. In addition, I was able to convert TRPV2 (which is insensitive to capsaicin) to become vanilloid sensitive with minimal mutations within the binding pocket, which further gave evidence of key residues involved in capsaicin binding and activation. Thus, it is proposed that several TRP channels may possess the same mechanisms or 'machinery' for ligand binding and activation. With this, the ligand binding pocket is more defined which can aid in the development of analgesic medication.

a. Yang, F.*, **S. Vu***, V. Yarov-Yarovoy, and J. Zheng (2016) Rational design and validation of a vanilloid-sensitive TRPV2 ion channel, *Proceedings of the National Academy of Sciences, USA, E3657–E3666.* *These authors contributed equally to this work. •• *Highlighted by F1000Prime*

b. Y Dong*, Y Yin*, **S Vu***, F Yang, V Yarov-Yarovoy, Y Tian, J Zheng. (2019) A distinct structural mechanism underlies TRPV1 activation by piperine. *BBRC*. *These authors contributed equally to this work.

c. Y Yin*, Y Dong*, **S Vu***, F Yang, V Yarov-Yarovoy, Y Tian, J Zheng. (2019) Structural Mechanism Underlying TRPV1 Activation by Pungent Compounds in Gingers. *British Journal of Pharmacology*. *These authors contributed equally to this work.

d. **Simon Vu**, Vikrant Singh, Heike Wulff, Vladimir Yarov-Yarovoy, Jie Zheng. (2020) New capsaicin analogs as molecular rulers to define the permissive conformation of the mouse TRPV1 ligand binding pocket. *eLife.*

e. Shisheng Li, Phuong T Nguyen, **Simon Vu**, Vladimir Yarov-Yarovoy, Jie Zheng. (2023) Opening of capsaicin receptor TRPV1 is stabilized equally by its four subunits. *Journal of Biological Chemistry.*

2. TRPV1 channel undergoes wide-spread conformational changes during polymodal activation of the channel: During my graduate research, I investigated the molecular mechanisms underlying polymodal activation of nociceptor and heat sensor TRPV1. Using the knowledge and skills acquired from previous work, we aimed to study how TRPV1 responds to multiple stimuli with exquisite sensitivity. Our goal was to reveal fundamental mechanisms for polymodal activation shared by several TRP channels, of which TRPV1 served as a model. In addition, I expanded my experimental tools using patch fluorometry with fluorescent noncanonical amino acids to understand the conformational changes that occur during ligand and heat activation of the channel. Using this novel technique, I determined that specified regions of TRPV1 are involved in different roles during activation. Previously, though the capsaicin-bound and apo structures of TRPV1 were resolved, the dynamic process of activation was not well understood. Through this work, we determined that channel activation is initiated at the intracellular side in the ligand binding pocket which then induces conformational changes in the outer pore region to open the channel. This provided insight on how the channel is activated via the outer pore by stimuli such as protons, heat, and animal toxins which bind to the pore turret. These discoveries shed light on TRPV1's activation mechanism and aid in developing compounds that activate TRPV1 through interactions with an alternate site and may prevent adverse effects such as hyperthermia.

a. Linlin Ma*, Fan Yang*, **Simon Vu**, Jie Zheng. (2015) Exploring functional roles of TRPV1 intracellular domains with unstructured peptide-insertion screening. *Equal Contribution. *Scientific Reports*.

b. Fan Yang, Xian Xiao, Bo Hyun Lee, **Simon Vu**, Wei Yang, Vladimir Yarov-Yarovoy, Jie Zheng. (2018) The Conformational Wave in Capsaicin Activation of Transient Receptor Potential Vanilloid 1 Ion Channel. *Nature Communications*.

c. A Zhu, A Aierken, Z Yao, **S Vu**, Y Tian, J Zheng, S Yang, F Yang. (2020) A centipede toxin causes rapid desensitization of nociceptor TRPV1 ion channel. *Toxicon*.

d. G Du, Y Tian, Z Yao, **S Vu**, J Zheng, L Chai. KW Wang, F Yang. (2020) A specialized pore turret in the mammalian cation channel TRPV1 is responsible for distinct and species-specific heat activation thresholds. *Journal of Biological Chemistry*.

3. Importance of TRP channels in physiology and disease: Polymodal TRP channels are important sensors in normal physiological signal transduction and pain, making them attractive drug targets. During my graduate research, I applied our improved mechanistic understanding of TRP channels to identify their new physiological roles. Investigation in this area incorporates multidisciplinary approaches and covers a wide range of biological questions about the role of these TRP channels as cellular sensors. I was able to delineate the mechanism of action of novel TRPA1 activators in a new binding site which was found to be the same as the capsaicin binding site of TRPV1. In addition, I helped determine a mechanism by which a mutation in TRPM4 led to over-activation of this channel causing Progressive Symmetric Erythrokeratoderma in patients.

a. Huijun Wang*, Zhe Xu*, Bo Hyun Lee*, **Simon Vu***, Linghan Hu, Mingyang Lee, Dingfang Bu, Xu Cao, Yong Yang, Jie Zheng, Zhimiao Lin. (2018) Gain-of-function Mutations in TRPM4 Activation Gate cause Progressive Symmetric Erythrokeratoderma. *Journal of Investigative Dermatology*. *These authors contributed equally to this work.

b. C Liu*, R Reese*, **S Vu*** *et al.* (2021) A non-covalent ligand reveals biased agonism of the TRPA1 ion channel. *Neuron*. *These authors contributed equally to this work.

c. Daisuke Yamada, **Simon Vu**, Xuesong Wu, Zhenrui Shi, Desiree Morris, Joshua D Bloomstein, Mindy Huynh, Jie Zheng, Samuel T Hwang. (2022) Gain-of-function Mutations of TRPM4 predisoses mice to psoriasiform dermatitis. *Frontiers In Immunology*.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/simon.vu.1/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: Kirichok, Yuriy Victorovich

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

POSITION TITLE: 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
Moscow Institute of Physics and Technology; Moscow, Russia	B.Sc.	09/1991-06/1995	Physics, Biophysics
Moscow Institute of Physics and Technology; Moscow, Russia	M.Sc.	09/1995-06/1997	Biophysics
Moscow Institute of Physics and Technology and Bogomoletz Institute of Physiology; Kiev, Ukraine	Ph.D.	09/1997-10/2000	Biophysics
Harvard Medical School, Children's Hospital Boston, and Howard Hughes Medical Institute; Boston, MA	Postdoctoral training	11/2000-11/2006	Biophysics and Physiology

A. Personal Statement

My lab pushes the boundaries of organellar physiology and biophysics, addressing long-standing biological questions that have resisted resolution for decades. We made groundbreaking contributions to several key areas, including: 1) the electrophysiological identification of the mitochondrial Ca^{2+} channel (MCU) that regulates ATP production; 2) the molecular and electrophysiological characterization of the Ca^{2+} channel (CatSper) essential for sperm flagellar motility; 3) the discovery of the sperm flagellar progesterone receptor (CatSper/ABHD2); and 4) the molecular identification and mechanistic understanding of the mitochondrial H^+ leak (mediated by AAC and UCP1) responsible for thermogenesis.

Resolving these complex questions requires years of dedicated research and effort. Much of our success has been driven by our development and application of advanced electrophysiological techniques, enabling direct recording of currents across both flagellar and mitochondrial membranes. Our pioneering efforts have gained significant recognition in bioenergetics and reproductive biology, and scientists from around the world frequently come to our lab to learn these electrophysiological methods for their own research.

Our current research focuses on the molecular physiology of mitochondrial membranes, a crucial area given the link between mitochondrial dysfunction and various diseases. However, the lack of detailed molecular insights into mitochondrial structure has hindered therapeutic advances. We are now at a critical point where further progress depends on a deeper understanding of the ultrastructure of mitochondrial membranes and its impact on bioenergetics. A principal challenge lies in preserving the native conformation of the mitochondrial membranes—including the cristae folds and inner-outer membrane connections—during sample preparation for cryo-ET and cryo-EM. This joint effort of the Fu and Kirichok labs aims to overcome these technical challenges. By applying structural biology to intact mitochondrial membranes, we hope to advance our goal of characterizing the primary anionic and cationic conductances of the inner mitochondrial membrane.

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

R35GM136415 (PI: Kirichok) 05/1/2020 - 04/30/2025
NIH/NIGMS
Molecular Biophysics of Mitochondrial Membranes

In this project, we are studying the molecular mechanisms of the mitochondrial H^+ leak and thermogenesis. We also are addressing structure–function relationships within the mitochondrial Ca^{2+} uniporter (MCU) ion channel complex.

1R01GM134536 (PI: Kirichok) 09/01/2019–06/30/2021
NIH/NIGMS
Molecular Mechanisms of Mitochondrial Ca^{2+} uptake
This project addressed structure–function relationships within the mitochondrial Ca^{2+} uniporter (MCU) ion channel complex. Specifically, the molecular mechanisms of ion permeation, ion selectivity and gating were studied.

R01GM118939 (PI: Kirichok) 03/01/2017 – 12/31/2021
NIH/NIGMS
Molecular Mechanisms of Mitochondrial Uncoupling and Thermogenesis
This project identified and provided functional characterization of the transport proteins of the inner mitochondrial membrane responsible for H^+ leak, mitochondrial uncoupling, and thermogenesis in tissues other than brown fat.

a. Bertholet AM, Kazak L, Chouchani ET, Bogaczyńska MG, Paranjpe I, Wainwright GL, Bétourné A, Kajimura S, Spiegelman BM, Kirichok Y. Mitochondrial Patch Clamp of Beige Adipocytes Reveals UCP1-Positive and UCP1-Negative Cells Both Exhibiting Futile Creatine Cycling. ***Cell Metabolism*** 2017; 25: 811-822, PMCID: PMC5448977

b. Bertholet AM, Chouchani ET, Kazak L, Angelin A, Fedorenko A, Long JZ, Vidoni S, Garrity R, Cho J, Terada N, Wallace DC, Spiegelman BM, and Kirichok Y. H^+ Transport is an Integral Function of the Mitochondrial ADP/ATP Carrier, ***Nature*** 2019; 571:515-520

c. Garg, V, Paranjpe I, Unsulangi T, Suzuki J, Milescu LS, Kirichok Y. The Mechanism of MICU-Dependent Gating of the Mitochondrial Ca^{2+} Uniporter. ***eLife*** 2021, 10:e69312. doi: 10.7554/eLife.69312

d. Bertholet AM, Natale AM, Bisignano P, Suzuki J, Fedorenko A, J Hamilton, Tatiana Brustovetsky T, Kazak L, Garrity R, Chouchani ET, Brustovetsky N, Grabe M*, and Kirichok Y* (2022) Mitochondrial Uncouplers Induce Proton Leak by Activating AAC and UCP1. ***Nature*** 2022; 606:180-187

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

10/2022–present	Professor, Department of Biochemistry and Molecular Biophysics, Washington University in St. Louis; St. Louis, MO
09/2019–present	Chief Scientific Officer, Equator Therapeutics; Richmond, CA
07/2016–08/2022	Professor, Department of Physiology, University of California San Francisco; San Francisco, CA
07/2012–06/2016	Associate Professor, Department of Physiology, University of California San Francisco; San Francisco, CA
12/2006–06/2012	Assistant Professor, Department of Physiology, University of California San Francisco; San Francisco, CA
11/2000–12/2006	Postdoctoral Research Associate in the laboratory of Dr. David Clapham, Howard Hughes Medical Institute, Harvard Medical School, and Department of Cardiology at Children's Hospital Boston; Boston, MA
03/1996–11/2000	Specialist, Department of Cellular Membranology, Bogomoletz Institute of Physiology; Kiev, Ukraine

- *Ad hoc reviewer/editor for journals and study sections:* I regularly review papers for scientific journals such as eLife, Science, Nature, Journal of Cell Biology, Nature Chemical Biology, New England Journal

of Medicine, Journal of General Physiology, and Biology of Reproduction. For PNAS, I have served as both ad hoc reviewer and editor. I have also served as an ad hoc grant reviewer for NIH and the American Heart Association.

- *Training other labs in advanced electrophysiology methods:* We pioneered the application of the patch-clamp technique to record currents mediated by ion channels and transporters across the whole inner mitochondrial membrane and the membrane of the sperm flagellum. These methods became indispensable tools in the fields of bioenergetics and sperm physiology, correspondingly. We routinely train in our lab as well as advise remotely scientists from research institutions around the world in these methods to facilitate their broad use and correct application.
- *Cofounder, Chief Scientific Officer, Equator Therapeutics.* Equator Therapeutics is a biotechnology company that is developing a drug to activate mitochondrial thermogenesis. The drug will increase body energy expenditure without exercise for the treatment of obesity, type 2 diabetes, and other metabolic disorders.

Honors

2013	Byers Award (UCSF Program for Breakthrough Biomedical Research)
2010	John S. Spice Award in Aging (Larry L. Hillblom Foundation)
2008	NIH Director's New Innovator Award
2008	Alfred P. Sloan Foundation Research Fellowship
2007	UCSF Jack D. and DeLoris Lange Endowed Chair in Systems Physiology
2007	Larry L. Hillblom Foundation Start-Up Research Grant

- *National/International invited talks*: I have been invited to present the results of our research at leading universities and research institutions including the University of California Berkeley, Harvard University, UT Southwestern Medical Center, NIH, University of Utah, Yale University, University of California Davis, New York University (NYU), University of Massachusetts Worchester, University of Iowa, University of Michigan, University of Osnabruck (Germany), Center of Advanced European Studies and Research (CAESAR)/Max Plank Institute (Germany), University of Tokyo (Japan), Novartis Institutes for Biomedical Research, and Washington University St. Louis.

- *Speaker/Organizer at scientific meetings*: I am regularly invited to speak at international scientific meetings such as Bioenergetics Subgroup Symposiums at Biophysical Society meetings, Keystone Symposia on Beige/Brown Fat, Society of General Physiologists meetings, European Bioenergetics Conference (EBEC) meetings, and Gordon Research Conferences on Membrane Transport Proteins, Ion Channels, Organellar Channels/Transporters, and Mitochondria. In addition, I co-organized a Symposium on Mitochondrial Ion Channels as a part of the 58th Annual Meeting of the Biophysical Society.

C. Contribution to Science

1. Development of advanced electrophysiological methods to study mitochondria and sperm cells.

Transport of ions and metabolites across the inner mitochondrial membrane (IMM) is the foundation of mitochondrial physiology, and the lack of a method to directly study such transport once was a major barrier in bioenergetics. In 2004, as a postdoc in David Clapham's lab at Harvard, I succeeded in applying the patch-clamp technique to record currents across the whole IMM of COS-7 cells and characterized the mechanism of mitochondrial Ca^{2+} uptake. Since I started my own lab at UCSF in 2006, we have improved this method by substantially increasing its success rate, while also making this technique applicable to mitochondria of any species/tissue and measuring activities of key mitochondrial transport proteins involved in mitochondrial energy conversion.

Sperm ion channels control essential milestones of the sperm's journey toward the egg, including activation of sperm motility, functional maturation in the female reproductive tract, chemotaxis toward the egg, and hyperactivation in the egg's vicinity. However, for several decades, numerous attempts to patch-clamp spermatozoa to identify and characterize sperm ion channels were frustrated by the small size, motility, and rigid architecture of sperm. While a postdoctoral fellow in the Clapham lab, I developed a method for applying

the patch-clamp technique to mouse sperm cells and recorded ion channel activity from the sperm flagellum. In 2008, in my own lab, we applied the patch-clamp technique to significantly smaller human sperm cells.

These electrophysiological methods have significantly pushed the boundaries of what was possible in their corresponding fields and resulted in transformative discoveries. These methods have been adopted by many laboratories around the world, either independently or with our help, and have become a new standard in studying mitochondrial and sperm ion channels/transporters. I developed the methods for mitochondrial and sperm patch-clamp while a postdoctoral fellow in the Clapham lab at Harvard Medical School and further improved them my own lab at UCSF. No collaborating investigators were involved.

a. Kirichok Y, Krapivinsky G, Clapham DE. The mitochondrial Ca^{2+} uniporter is a highly selective channel. ***Nature*** 2004; 427: 360-4.

b. Fedorenko A, Lishko PV, Kirichok Y. Mechanism of fatty acid-dependent UCP1 uncoupling in brown fat mitochondria. ***Cell*** 2012; 151: 400-13. PMCID: PMC3782081

c. Kirichok Y, Navarro B, Clapham DE. Whole-cell patch clamp measurements of spermatozoa reveal an alkaline-activated Ca^{2+} channel. ***Nature*** 2006; 439: 737-40.

d. Lishko PV, Botchkina IL, Fedorenko A, Kirichok Y. Acid extrusion from human spermatozoa is mediated by flagellar voltage-gated proton channel. ***Cell*** 2010; 140; 327-37. PMID: 20144758

2. Transformative discoveries in sperm physiology and male fertility.

Sperm ion channels control sperm motility and fertility by regulating sperm intracellular [Ca^{2+}] and pH. Using sperm patch-clamp, we identified the sperm ion channels involved in the control of intracellular Ca^{2+} and pH and laid a foundation for understanding sperm physiology on the molecular level. First, we identified a pH-gated sperm Ca^{2+} channel that sets intracellular [Ca^{2+}] and demonstrated that it is formed by sperm-specific proteins CatSper1–4. Second, we identified the voltage-gated H^+ channel Hv1 as the principal H^+ channel of human spermatozoa that sets intracellular pH and controls the CatSper channel. Third, we provided electrophysiological identification and characterization of the principal sperm K^+ channel (KSper) that determines the sperm membrane potential and regulates CatSper and Hv1 activity. Finally, we demonstrated that the human CatSper channel is associated with a membrane non-genomic receptor for the female steroid hormone progesterone. Through progesterone-activated CatSper, the motility and fertility of human spermatozoa are dependent on the female reproductive cycle, which helps them to fertilize the egg. Importantly, the CatSper channel and associated progesterone receptor have become major targets for controlling male fertility. Identification of the molecular composition of the CatSper channel and electrophysiological identification of the KSper channel were achieved during my postdoctoral training in the Clapham lab in collaboration with other postdocs. Research identifying Hv1 and the CatSper-associated non-genomic progesterone receptor was performed in my own lab.

a. Kirichok Y, Navarro B, Clapham DE. Whole-cell patch clamp measurements of spermatozoa reveal an alkaline-activated Ca^{2+} channel. ***Nature*** 2006; 439: 737-40.

b. Lishko PV, Botchkina IL, Fedorenko A, Kirichok Y. Acid extrusion from human spermatozoa is mediated by flagellar voltage-gated proton channel. ***Cell*** 2010; 140; 327-37. PMID: 20144758

c. Lishko PV, Botchkina IL, Kirichok Y. Progesterone activates the principal Ca^{2+} channel of human sperm. ***Nature*** 2011; 471: 387-91.

d. Smith JF, Syritsyna O, Fellous M, Serres C, Mannowetz N, Kirichok Y*, and Lishko PV*. Disruption of the principal, progesterone-activated sperm Ca^{2+} channel in a CatSper2-deficient infertile patient. ***Proc Natl Acad Sci USA*** 2013; 110: 6823-8; PMCID: PMC3637729; *- co-corresponding authors

3. Electrophysiological identification and characterization of the mitochondrial Ca^{2+} uniporter (MCU), the channel responsible for the phenomenon of mitochondrial Ca^{2+} uptake. Mitochondria accumulate Ca^{2+} during intracellular Ca^{2+} transients. This phenomenon, known as mitochondrial Ca^{2+} uptake, is responsible for stimulating mitochondrial energy production and shaping intracellular Ca^{2+} signals, and has been implicated in initiating necrotic and apoptotic cell death. Mitochondrial Ca^{2+} uptake was first studied indirectly using ion flux assays in suspensions of isolated mitochondria and with Ca^{2+} imaging in intact cells. Although these studies established the phenomenon of mitochondrial Ca^{2+} uptake, the identity of the transport mechanism responsible (referred to as the mitochondrial Ca^{2+} uniporter, MCU) remained elusive for many decades. In 2004, while a postdoctoral fellow in the Clapham lab, I applied the patch-clamp technique to the whole inner membrane of COS-7 mitochondria and demonstrated that the MCU is a novel, highly selective Ca^{2+} ion channel and determined its functional properties. In my own lab, we compared the MCU current density in mitochondria of various mouse tissues. Unexpectedly, it varied very broadly between tissues, with

the MCU current density in heart mitochondria being about 30 times lower than that in skeletal muscle. This discovery changed the perception of MCU as a molecule with an essential ("housekeeping") role and demonstrated that the amount of MCU activity is tightly controlled in various tissues to prevent derangement of intracellular Ca^{2+} signaling and mitochondrial Ca^{2+} overload. The electrophysiological identification and characterization of MCU laid a foundation for its further molecular characterization.

a. Kirichok Y, Krapivinsky G, Clapham DE. The mitochondrial Ca^{2+} uniporter is a highly selective channel. ***Nature*** 2004; 427: 360-4.

b. Fieni F, Bae Lee S, Jan YN, Kirichok Y. Activity of the mitochondrial calcium uniporter varies greatly between tissues. ***Nature Commun*** 2012; 3: 1317. doi: 10.1038/ncomms2325; PMCID: PMC3818247

c. Fieni F, Johnson DE, Hudmon A, and Kirichok Y. Mitochondrial Ca^{2+} uniporter and CaMKII in heart. ***Nature*** 2014; 513: E1–E2. doi:10.1038/nature13626. PMCID: PMC4476531

d. Garg, V, Paranjpe I, Unsulangi T, Suzuki J, Milescu LS, Kirichok Y. The Mechanism of MICU-Dependent Gating of the Mitochondrial Ca^{2+} Uniporter. ***eLife*** 2021, 10:e69312. doi: 10.7554/eLife.69312

4. Elucidation of the mechanisms of mitochondrial H^+ leak and thermogenesis.

Mitochondrial uncoupling proteins (UCPs) make the inner mitochondrial membrane permeable for H^+ in the presence of free fatty acids (FA). This so-called "H^+ leak" decreases coupling between the electron transport chain and the ATP synthase, reduces the efficiency of ATP production and results in dissipation of heat (mitochondrial thermogenesis). Activation of H^+ leak via UCPs has emerged as a promising target for treating obesity, diabetes, mitochondrial oxidative stress, and age-related disorders. However, the molecular mechanisms of the mitochondrial H^+ leak were poorly understood. Although UCP1 had long been established as the UCP of the specialized thermogenic tissue brown fat, the mechanism by which FA activate H^+ leak via UCP1 remained controversial. Moreover, the UCP(s) of all other tissues were elusive.

We succeeded in measuring H^+ leak carried by UCP1 directly from the whole IMM of brown fat using the patch-clamp technique. We found that FA activate the H^+ leak via UCP1 by serving as its transport substrates, and UCP1 operates as an unusual FA anion/H^+ symporter. We also measured mitochondrial H^+ leak in beige adipocytes (a recently identified thermogenic adipocytes in white fat depots). Surprisingly, we discovered two distinct types of beige fat cells, UCP1-positive and UCP1-negative. Although UCP1-negative beige adipocytes lack the UCP1-dependent H^+ leak, we demonstrated that they possess an alternative thermogenic mechanism—a futile cycle of creatine phosphorylation/dephosphorylation. In our most recent research on mitochondrial uncoupling, we directly measured the mitochondrial H^+ leak in tissues such as skeletal muscle, heart, liver, and kidney (that do not express UCP1). We demonstrated that the mitochondrial ADP/ATP carrier (AAC), which is responsible for export of ATP into cytosol in exchange for cytosolic ADP, also mediates FA-dependent H^+ leak in these tissues (Nature 2019). Finally, we also demonstrated that common mitochondrial uncouplers such as DNP and FCCP activate H^+ leak via AAC and UCP1. Activation of these two uncoupling proteins, and not protonophoric activity, is the primary mechanism by which these two compounds induce mitochondrial uncoupling (Nature 2022). These findings transformed our understanding of the molecular mechanisms of mitochondrial H^+ leak and now enable rational development of tools for its pharmacological control.

a. Fedorenko A, Lishko PV, Kirichok Y. Mechanism of fatty acid-dependent UCP1 uncoupling in brown fat mitochondria. ***Cell*** 2012; 151: 400-13. PMCID: PMC3782081

b. Bertholet AM, Kazak L, Chouchani ET, Bogaczyńska MG, Paranjpe I, Wainwright GL, Bétourné A, Kajimura S, Spiegelman BM, Kirichok Y. Mitochondrial Patch Clamp of Beige Adipocytes Reveals UCP1-Positive and UCP1-Negative Cells Both Exhibiting Futile Creatine Cycling. ***Cell Metabolism*** 2017; 25: 811-822, PMCID: PMC5448977

c. Bertholet AM, Chouchani ET, Kazak L, Angelin A, Fedorenko A, Long JZ, Vidoni S, Garrity R, Cho J, Terada N, Wallace DC, Spiegelman BM, and Kirichok Y. H^+ Transport is an Integral Function of the Mitochondrial ADP/ATP Carrier, ***Nature*** 2019; 571:515-520

d. Bertholet AM, Natale AM, Bisignano P, Suzuki J, Fedorenko A, J Hamilton, Tatiana Brustovetsky T, Kazak L, Garrity R, Chouchani ET, Brustovetsky N, Grabe M*, and Kirichok Y* (2022) Mitochondrial Uncouplers Induce Proton Leak by Activating AAC and UCP1. ***Nature*** 2022; 606:180-187

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/yuriy.kirichok.1/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: Fu, Ziao

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

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
Jilin University, Changchun, China	B.S.	05/2012	Chemistry
Stony Brook University, Stony Brook, NY	M.S.	05/2014	Chemistry
Columbia University, New York, NY	Ph.D.	05/2019	Biological Science
Rockefeller University, New York, NY	Postdoctoral	09/2024	Molecular Neurobiology and Biophysics

A. Personal Statement

I am an Assistant Professor of Cell Biology & Physiology at Washington University in St. Louis, specializing in using cryo-electron microscopy (cryo-EM) to study membrane protein structures in their native environments. This proposal focuses on resolving the high-resolution structure of native mitochondria-associated ribosomes to understand their spatial organization, interactions, and functional dynamics. My research aims to reveal how the structural and spatial properties of ribosomes contribute to their role in mitochondrial translation and energy production, as well as their regulation in health and disease. I have extensive expertise in cryo-EM, developed during my Ph.D. with Dr. Joachim Frank at Columbia University, where I studied ribosome structures and contributed to understanding their dynamics and functional states. This experience was further refined as a postdoctoral researcher in Dr. Rod MacKinnon's lab, where I pioneered methods to study membrane proteins directly in their native lipid bilayers. These approaches preserve crucial protein-lipid interactions and enable high-resolution structural determination. My background has uniquely prepared me to investigate native mitochondria associated ribosome structures, preserving their physiological interactions with mitochondrial membranes and accessory proteins. Our lab specializes in solving protein structures from native membrane environments, and we have successfully applied similar workflows to other membrane-associated complexes. Leveraging this expertise, we aim to use cryo-EM to investigate mitochondria-associated ribosomes under varying physiological conditions, such as normal, stressed, and hibernated states. The advanced resources at the Washington University Center for Cellular Imaging (WUCCI), including state-of-the-art Titan Krios microscopes and computational infrastructure, position us ideally to achieve our research objectives. Utilizing NCCAT's cutting-edge cryo-EM facility will further enhance data collection efficiency and support our goal of achieving high-resolution maps that uncover the mechanistic details of ribosome function and regulation. This study will provide foundational insights into mitochondrial protein synthesis, its dysregulation in diseases such as mitochondrial myopathies and neurodegenerative disorders, and potential avenues for therapeutic intervention. With WUSTL's collaborative environment and my lab's expertise, I am well-positioned to lead this study and generate impactful insights into the structural biology of mitochondria-associated ribosomes.

Citations:

a. Feng, X.*; **Fu, Z.***; Kaledhonkar, S.; Jia, Y.; Shah, B.; Jin, A.; Liu, Z.; Sun, M.; Chen, B.; Grassucci, R. A.; Ren, Y.; Jiang, H.; Frank, J.; Lin, Q. A Fast and Effective Microfluidic Spraying-Plunging Method

for High-Resolution Single-Particle Cryo-EM. Structure 2017, 25 (4), 663-670.e3. https://doi.org/10.1016/j.str.2017.02.005. PMCID: PMC5382802

b. **Fu, Z.***; Indrisiunaite, G.*; Kaledhonkar, S.*; Shah, B.; Sun, M.; Chen, B.; Grassucci, R. A.; Ehrenberg, M.; Frank, J. The Structural Basis for Release-Factor Activation during Translation Termination Revealed by Time-Resolved Cryogenic Electron Microscopy. Nat. Commun. 2019, 10 (1), 2579. PMCID: PMC6561943

c. Kaledhonkar, S.*; **Fu, Z.***; Caban, K.*; Li, W.; Chen, B.; Sun, M.; Gonzalez, R. L.; Frank, J. Late Steps in Bacterial Translation Initiation Visualized Using Time-Resolved Cryo-EM. Nature 2019, 570 (7761), 400–404. PMCID: PMC7060745

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

10/2024 - Assistant Professor, Department of Cell Biology & Physiology, Washington University, St. Louis, MO

Honors

2019 Titus M. Coan Prize for Excellence in Research (Basic Cell and Molecular Biology)
2019 Best Poster Award The 7th International Ion Channel Conference
2018 COMPPÅ Symposium Fisher Award

Professional Societies and Organizations

2014 - Biophysical Society

C. Contributions to Science

1. My early graduate research focused on the aggregation of amyloid-β (Aβ) peptides, a key process in Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA). Using advanced biophysical techniques, I discovered that Aβ initially forms unstructured low-molecular-weight oligomers that stack into more toxic forms, leading to protofibril formation. Importantly, I found that curcumin and resveratrol, natural compounds from curry and red wine, can bind to these early oligomers, preventing their toxic progression. Additionally, I demonstrated that familial mutations in Aβ peptides, linked to early-onset AD and CAA, lead to unique structural intermediates and that preexisting amyloid can influence the aggregation of Aβ monomers into disease-specific structures. These findings provide crucial insights into the molecular mechanisms of these neurodegenerative diseases and suggest new therapeutic targets. My role encompassed designing and conducting experiments, as well as interpreting the data to connect these structural changes to disease pathology.

a. **Fu, Z.**; Aucoin, D.; Ahmed, M.; Ziliox, M.; Van Nostrand, W. E.; Smith, S. O. Capping of Aβ42 Oligomers by Small Molecule Inhibitors. Biochemistry 2014, 53 (50), 7893–7903. PMCID: PMC4278677

b. **Fu, Z.**; Aucoin, D.; Davis, J.; Van Nostrand, W. E.; Smith, S. O. Mechanism of Nucleated Conformational Conversion of Aβ42. Biochemistry 2015, 54 (27), 4197–4207. PMID: 26069943

c. Xu, F.*; **Fu, Z.***; Dass, S.; Kotarba, A. E.; Davis, J.; Smith, S. O.; Van Nostrand, W. E. Cerebral Vascular Amyloid Seeds Drive Amyloid β-Protein Fibril Assembly with a Distinct Anti-Parallel Structure. Nat. Commun. 2016, 7 (1), 13527. PMCID: PMC5121328

d. **Fu, Z.***; Crooks, E. J.*; Irizarry, B. A.; Zhu, X.; Van Nostrand, W. E.; Chowdury, S.; Smith, S. O. An Electrostatic Cluster Guides Aβ40 Fibril Formation in Cerebral Amyloid Angiopathy. J. Struct. Biol. 2024 Apr 13;216(2):108092. PMCID: PMC11162928

2. The study of short-lived intermediates in biological processes, such as bacterial translation, has historically been limited by the inability to capture these fleeting states at high resolution. In 2014, during my Ph.D. research in Joachim Frank's lab at Columbia University, I focused on optimizing time-resolved cryo-electron microscopy (cryo-EM) to overcome this challenge. My work centered on capturing and analyzing intermediate states during key stages of bacterial translation, including ribosome recycling, translation initiation, and termination. The central findings of my research were the discovery and high-resolution characterization of previously unknown intermediate states in these processes. These discoveries provided critical insights into the molecular mechanisms of translation and have significant implications for the development of new antibiotics. My role involved the technical optimization of the time-resolved cryo-EM method, designing

experiments, and interpreting the structural data, which collectively advanced the application of this powerful technique to broader biological questions.

a. **Fu, Z.***; Kaledhonkar, S.*; Borg, A.*; Sun, M.; Chen, B.; Grassucci, R. A.; Ehrenberg, M.; Frank, J. Key Intermediates in Ribosome Recycling Visualized by Time-Resolved Cryoelectron Microscopy. Structure 2016, 24 (12), 2092–2101. PMCID: PMC5143168

b. Feng, X.*; **Fu, Z.***; Kaledhonkar, S.; Jia, Y.; Shah, B.; Jin, A.; Liu, Z.; Sun, M.; Chen, B.; Grassucci, R. A.; Ren, Y.; Jiang, H.; Frank, J.; Lin, Q. A Fast and Effective Microfluidic Spraying-Plunging Method for High-Resolution Single-Particle Cryo-EM. Structure 2017, 25 (4), 663-670.e3. https://doi.org/10.1016/j.str.2017.02.005. PMCID: PMC5382802

c. **Fu, Z.***; Indrisiunaite, G.*; Kaledhonkar, S.*; Shah, B.; Sun, M.; Chen, B.; Grassucci, R. A.; Ehrenberg, M.; Frank, J. The Structural Basis for Release-Factor Activation during Translation Termination Revealed by Time-Resolved Cryogenic Electron Microscopy. Nat. Commun. 2019, 10 (1), 2579. PMCID: PMC6561943

d. Kaledhonkar, S.*; **Fu, Z.***; Caban, K.*; Li, W.; Chen, B.; Sun, M.; Gonzalez, R. L.; Frank, J. Late Steps in Bacterial Translation Initiation Visualized Using Time-Resolved Cryo-EM. Nature 2019, 570 (7761), 400–404. PMCID: PMC7060745

3. The activation mechanisms of Class-C G protein-coupled receptors (GPCRs) have long been a challenging area in biomedical research due to their complex structures and roles in various physiological processes. During my Ph.D. training, I collaborated with Dr. Qing R. Fan, a leading expert in Class-C GPCRs at Columbia University, to address these challenges. My research focused on elucidating the structures of key Class-C GPCRs in different functional states. I successfully determined the structure of the human calcium-sensing receptor (CaSR) in its active state, uncovering symmetric activation and modulation mechanisms with significant implications for calcium-related therapies. Additionally, I characterized the structure of the human GABAB receptor in its inactive state, providing crucial insights for the development of drugs aimed at treating neurological disorders such as epilepsy and depression. These findings advance our understanding of Class-C GPCR activation and regulation, opening new avenues for therapeutic interventions in both calcium-related and neurological diseases. My specific role in this work included designing experiments, solving the receptor structures, and interpreting the data to reveal the mechanisms underlying their activation and regulation.

a. Park, J.*; **Fu, Z.***; Frangaj, A.*; Liu, J.*; Mosyak, L. *; Shen, T. *; Slavkovich, V. N.; Ray, K. M.; Taura, J.; Cao, B.; Geng, Y.; Zuo, H.; Kou, Y.; Grassucci, R.; Chen, S.; Liu, Z.; Lin, X.; Williams, J. P.; Rice, W. J.; Eng, E. T.; Huang, R. K.; Soni, R. K.; Kloss, B.; Yu, Z.; Javitch, J. A.; Hendrickson, W. A.; Slesinger, P. A.; Quick, M.; Graziano, J.; Yu, H.; Fiehn, O.; Clarke, O. B.; Frank, J.; Fan, Q. R. Structure of Human GABAB Receptor in an Inactive State. Nature 2020, 584 (7820), 304–309. PMCID: PMC7725281

b. Park, J.*; Zuo, H.*; Frangaj, A.*; **Fu, Z.***; Yen, L. Y.*; Zhang, Z.*; Mosyak, L.; Slavkovich, V. N.; Liu, J.; Ray, K. M.; Cao, B.; Vallese, F.; Geng, Y.; Chen, S.; Grassucci, R.; Dandey, V. P.; Tan, Y. Z.; Eng, E.; Lee, Y.; Kloss, B.; Liu, Z.; Hendrickson, W. A.; Potter, C. S.; Carragher, B.; Graziano, J.; Conigrave, A. D.; Frank, J.; Clarke, O. B.; Fan, Q. R. Symmetric Activation and Modulation of the Human Calcium-Sensing Receptor. Proc. Natl. Acad. Sci. 2021, 118 (51), e2115849118. PMCID: PMC8713963

4. The mechanosensitive ion channel Piezo1 plays a vital role in sensing membrane tension, yet understanding its structural mechanism in native-like environments has been a challenge. In 2019, I joined Rod MacKinnon's lab to address this problem, leveraging my expertise in cryo-electron microscopy (cryo-EM) to study membrane proteins in more native settings. Previous studies showed that Piezo1, when stabilized in detergents, exhibited a highly curved structure, but how this related to its function within a natural lipid bilayer was unclear. To explore this, I reconstituted Piezo1 into liposomes, which more accurately simulate the native lipid bilayer environment. My central finding was that Piezo1 induces tear-drop shapes in spherical liposomes, with the surrounding curved membrane exerting forces on the channel. By analyzing vesicles of varying sizes, I demonstrated that Piezo1 adopts different shapes based on the membrane forces, allowing for the calculation of its stiffness. Using cryogenic electron tomography (cryo-ET), I obtained precise 3D shapes of Piezo1 vesicles and discovered that Piezo1 is less curved in planar lipid bilayers than in detergent micelles, revealing that its rigidity is comparable to that of a free lipid bilayer. These insights provide a crucial framework for understanding how proteins deform bilayer membranes and enhance our understanding of Piezo1's mechanosensory function. My specific role involved reconstituting Piezo1 in liposomes, conducting cryo-EM

and cryo-ET analyses, and interpreting the data to elucidate the structural dynamics of Piezo1 in its native-like environment.

a. Haselwandter, C. A.; Guo, Y. R.; **Fu, Z.**; MacKinnon, R. Quantitative Prediction and Measurement of Piezo's Membrane Footprint. Proc. Natl. Acad. Sci. 2022, 119 (40), e2208027119. PMCID: PMC9546538

b. Haselwandter, C. A.; Guo, Y. R.; **Fu, Z.**; MacKinnon, R. Elastic Properties and Shape of the Piezo Dome Underlying Its Mechanosensory Function. Proc. Natl. Acad. Sci. 2022, 119 (40), e2208034119. PMCID: PMC9546593

5. The formation of membrane microdomains, essential for various cellular processes such as signaling and trafficking, has been a complex area of study, particularly in understanding the role of the Flotillin complex. Historically, structural characterization of such membrane proteins has been hindered by the need for detergents, which can disrupt native protein-lipid interactions. In this study, I aimed to overcome these challenges by elucidating the structure of the Flotillin complex within its native membrane environment. Utilizing advanced cryo-electron microscopy combined with a novel membrane protein stabilization method, I successfully resolved the structure of the Flotillin complex without the use of detergents or overexpression, preserving its natural conformation and interactions with the surrounding lipid bilayer. The central finding of this research was the detailed architecture of the Flotillin complex, which provided new insights into its role in inducing membrane curvature and organizing microdomains. This understanding is crucial for elucidating how Flotillin proteins influence processes such as clathrin-independent endocytosis. The ability to study the Flotillin complex in its native state represents a significant advancement in membrane biology and paves the way for future research into the functional roles of SPFH family proteins and their implications in various cellular processes and disease mechanisms. My specific role in this work included designing the experimental approach, conducting cryo-EM analysis, and interpreting the data to uncover the structural dynamics of the Flotillin complex in its native environment.

a. **Fu, Z.**; MacKinnon, R. Structure of the Flotillin Complex in a Native Membrane Environment. Proc. Natl. Acad. Sci. 2024, 121 (29), e2409334121. PMCID: PMC11260169

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/ziao.fu.1/bibliography/public/