OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 01/31/2026)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Lishko, Polina

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

POSITION TITLE: Professor of Cell Biology and Physiology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date MM/YYYY	FIELD OF STUDY
National Taras Shevchenko University, Kyiv, Ukraine	BS & Specialist (equivalent to M.S); *Summa Cum Laude*	06/1996	Biology, Biochemistry
The National Academy of Sciences of Ukraine, Bogomoletz Institute of Physiology, Kyiv, Ukraine.	Ph.D.	02/2000	Biophysics, Neuroscience
Harvard Medical School/Massachusetts Eye and Ear Infirmary, Boston, MA	Postdoctoral	06/2005	Ophthalmology, Biochemistry
Harvard University, Cambridge, MA	Postdoctoral	11/2006	Molecular and Cellular Biology, Protein Structure
University of California, San Francisco, CA	Specialist III (Instructor)	12/2011	Physiology, and Reproductive Biology

A. Personal Statement

I have extensive experience in ion channel physiology (over 27 years), neuroscience and endocrinology (14+ years), reproductive biology and aging (16+ years), and ophthalmology (5+ years). My team studies steroid signaling and its regulation of mammalian metabolism, aging, and reproduction through membrane steroid receptors and ion channels.

I received my Ph.D. in Biophysics from the National Academy of Sciences of Ukraine in 2000, where I studied the molecular mechanisms of neurodegeneration and memory. My initial postdoctoral training took place at Harvard Medical School in 2000, followed by Harvard University, where I worked on the molecular mechanisms of light perception in the eye and examined the structure-function relationship of heat-activated TRPV ion channels, which serve as receptors for pain and temperature.

From 2006 to 2011, as an instructor at the University of California, San Francisco, I studied the role of ion channels in reproduction and mitochondrial ion transporters in thermogenesis. In 2012, I joined the faculty at the University of California, Berkeley, where my team investigated how mammalian reproduction and neuronal functions are regulated by unconventional steroid signaling. In 2016, we identified α/β hydrolase domain–containing protein 2 (ABHD2) as a novel progesterone receptor, solving the decades-long puzzle of the molecular mechanism behind progesterone signaling in male gametes (Miller et al., Science 2016).

Recently, we discovered another non-genomic molecular target of progesterone—an inwardly rectifying potassium channel, Kir7.1, a crucial protein for the function of the choroid plexus, retinal pigment epithelium, and myometrial quiescence (Björkgren et al., J of Gen Physiology 2021). Using both reproductive and epithelial tissues, my lab has demonstrated how their physiology is regulated by steroid hormones, involving ion channels and membrane receptors.

In 2023, I moved my lab to Washington University in St. Louis, School of Medicine, where I assumed the role of BJC Investigator in the Department of Cell Biology and Physiology. Since then, my lab has collaborated with Professor Douglas Cover's lab on the role of steroid regulation in Kir7.1, where we identified several new Kir7.1 activators and inhibitors.

For years, my lab has been developing and applying advanced biophysical, biochemical, and cell biology techniques to investigate how steroid signaling regulates mammalian tissues and cells, particularly in the areas of reproduction, aging, and metabolism. Throughout my career, I have published over 50 peer-reviewed papers in leading scientific journals, including *Science, Nature, Cell, PNAS, Neuron, and Journal of General Physiology*, among others. My research accomplishments are well-recognized by professional societies, as reflected in the professional experience outlined below.

I have presented at more than 50 international, national, and local conferences, and have been invited to speak at numerous seminar series. I've also served on various NIH study sections and was a lecturer in the prestigious Frontiers in Reproduction course taught at the Marine Biological Laboratory in Woods Hole. Additionally, I have been part of NIH/NICHD study sections and serve on the editorial advisory boards for PLOS Biology, the Journal of General Physiology, and Bioelectricity.

Since 2012, I have mentored 10 graduate students, 13 postdoctoral fellows, and over 30 undergraduate students. Many of my trainees have been accepted into medical or graduate schools, received prestigious fellowships, or published high-impact research. Some have gone on to non-academic careers, including becoming program officers at the U.S. Department of Defense, supervisors at fertility clinics, or co-founders of successful startups such as YourChoice Therapeutics and Equator Therapeutics. I am also a co-founder of two companies: YourChoice Therapeutics and Biotock.

As a member of the several graduate programs as training faculty and principal investigator, I am fully committed to the responsible conduct of research, strict scientific ethics, thoughtful mentorship for my trainees, and the promotion of a safe, inclusive and supportive research training environment. This commitment includes my willingness to: 1) provide all of my mentees with training in rigorous and unbiased experimental design, methodology, analysis, interpretation, and reporting of results; 2) meaningfully participate in mentorship and unconscious bias training programs whenever required; and 3) ensure that my trainees meet requirement that thesis committee meetings are no more than 6 months apart. Finally, I recognize that an important part of my role as a mentor is to help all my trainees complete their degrees in a timely fashion with the skills, credentials, and experiences needed to sustain careers in the biomedical research workforce as productive research physicians.

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

R03 NIH AG-7-755-01
(multi-PI: Lishko, Garrison)
2/01/2021 – 12/31/2023
Steroid signaling in the choroid plexus of the aging brain

Bakar Prize Award
Lishko (PI)
6/01/2022 – 5/31/2025
Development of novel therapies to treat age-related macular degeneration and prevent blindness

Citations:

1. Björkgren I, Mendoza S, Chung DH, Haoui M, Petersen N, and Lishko PV. The epithelial potassium channel Kir7.1 is stimulated by progesterone. ***J of Gen Physiology.*** 2021. Oct 4;153(10):e202112924. doi: 10.1085/jgp.202112924. Epub 2021 Aug 13. PMID: 34387656
2. Björkgren I, Chung DH, Mendoza S, Gabelev-Khasin L, Modzelewski A, He L, and Lishko PV. Alpha/Beta Hydrolase Domain-Containing Protein 2 regulates the rhythm of follicular maturation and estrous stages of the female reproductive cycle. ***Front Cell Dev Biol.*** 2021, Sep 8; doi: 10.3389/fcell.2021.710864
3. Miller MR, Mannowetz N, Iavarone AT, Safavi R, Gracheva EO, Smith JF, Hill RZ, Bautista DM, Kirichok Y, Lishko P.V. Unconventional endocannabinoid signaling governs sperm activation via sex hormone progesterone. ***Science*** 2016 Apr 29;352(6285):555-9. doi: 10.1126/science. aad6887. Epub 2016 Mar 17 PMID: 26989199

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

01/2023- present	BJC Investigator; Professor of Cell Biology and Physiology at Washington University in St. Louis, School of Medicine
01/2023- 12/2023	Adjunct Associate Professor, Department of Molecular and Cellular Biology, University of California, Berkeley; Berkeley, CA
10/2019- 12/2022	Adjunct Associate Professor. The Center for Reproductive Longevity and Equality (CRLE) at Buck Institute for Research on Aging
07/2018- 12/2022	Associate Professor, Department of Molecular and Cellular Biology, University of California, Berkeley; Berkeley, CA
01/2012- 06/2018	Assistant Professor, Department of Molecular and Cellular Biology, University of California, Berkeley; Berkeley, CA
12/2006- 12/2011	Specialist III (Instructor) in the laboratory of Dr. Yuriy Kirichok at University of California, San Francisco; San Francisco, CA
08/2005–11/2006	Postdoctoral Research Associate in the laboratory of Dr. Rachelle Gaudet at Harvard University, Department of Molecular and Cellular Biology; Cambridge, MA
11/2000–06/2005	Postdoctoral Research Associate in the laboratory of Dr. Vadim Arshavsky at Harvard Medical School, Massachusetts Eye and Ear Infirmary, Department of Ophthalmology; Boston, MA
09/1993–11/2000	Research Associate, Department of Cellular Membranology, Bogomoletz Institute of Physiology, Kyiv, Ukraine

Honors

2023 Newsweek 50 American Medical Marvels Disrupting Healthcare
2022 “Great Immigrant, Great American” award by Carnegie Corporation of New York
2022 Bakar Prize (UC Berkeley)
2022 The Paul F. Cranefield Award from the Council of the Society of General Physiologists
2020 France-Berkeley Fund award
2020 Global Consortium for Reproductive Longevity and Equality Pilot Research award
2020 Pew Innovation Fund award
2020 MacArthur Fellowship award
2019 Chau Hoi Shuen Foundation Women in Science award
2018 Bakar Spark Fund award
2017 Matthew P. Hardy Young Andrologist award from American Society of Andrology
2017 Rose Hill Innovator award
2016 Margaret Oakley Dayhoff award from Biophysical Society
2015 Alfred P. Sloan Foundation Research Fellowship
2015 Pew Scholar award
2014 Hellman Family Fellowship
2013 March of Dimes Basil O'Connor Starter Scholar Research award
2012 Winkler Family Fellowship
2012 New and Notable Speaker at Biophysical Society Meeting, San Diego, CA
2003 Knights Templar Eye Foundation Fellowship
2001 *Fight for Sight/Prevent Blindness America* Fellowship

Other Experience and Professional Memberships

2019 - present	PLOS Biology. Editorial Board member
2019 - 2022	*eLife,* Member of the Board of Reviewing Editors
2018 - present	member of the American Society of Andrology Executive Council
2018 - present	The Society for the Study of Reproduction, member
2018 - present	*Journal of General Physiology* Editorial Advisory Board member
2018 - present	Ad hoc member, NIH/NICHD Study section/CMIR
2017 - present	Ad hoc member, NIH/NICHD Study section/U54 Contraceptive Centers
2012, 2016 - present	American Society of Andrology member

2014 - 2019	The American Society for Cell Biology, member
2012 - present	Ad hoc referee for Medical Research Council (MRC), UK. Reviewed chapters for new "Biology of Development" textbook, Garland Sciences/Taylor and Francis
2009 - present	Member, Biophysical Society
2006 - present	Ad hoc reviewer for: *Science, Development, eLife, Journal of Biological Chemistry, Journal of General Physiology, Nature Communications, Scientific Reports, Journal of Cellular Physiology, Biology of Reproduction, PLOS One, Molecular Human Reproduction, Human Reproduction, International Journal of Andrology, Molecular Reproduction and Development*.

C. Contributions to Science

1. **Expertise in ophthalmology and vision science, with a focus on the regulation of the phototransduction cascade in mammalian photoreceptors**. Made a key discovery on the molecular and structural mechanism of RGS9 activation of G-protein transducin, demonstrating that the N-terminal DEP domain of RGS9 is critical for its membrane association in photoreceptors. Co-authored a publication reporting the discovery of protein translocation between the outer and inner segments of photoreceptors, identifying it as a crucial mechanism for light adaptation in the mammalian retina.

a. Lishko PV, Martemyanov KA, Hopp JA, Arshavsky VY.J Biol Chem. Specific binding of RGS9-Gbeta 5L to protein anchor in photoreceptor membranes greatly enhances its catalytic activity. 2002 Jul 5;277(27):24376-81. doi: 10.1074/jbc.M203237200. Epub 2002 May 2.PMID: 12006596
b. Strissel KJ, Lishko PV, Trieu LH, Kennedy MJ, Hurley JB, Arshavsky VY. Recoverin undergoes light-dependent intracellular translocation in rod photoreceptors. J Biol Chem. 2005 Aug 12;280(32):29250-5. doi: 10.1074/jbc.M501789200. Epub 2005 Jun 16.PMID: 15961391

2. **Expertise in structural biology**. I determined the structure-function relationship of the TRPV1 ion channel, revealing how this protein is regulated by various endogenous ligands. In 2005, I joined Dr. Rachelle Gaudet's laboratory in the Molecular and Cell Biology Department at Harvard University. There, I successfully combined X-ray crystallography with my expertise in patch-clamp electrophysiology to investigate the structure-function relationships of temperature-activated ion channels (TRP channels). This work led to several publications, including a Neuron paper in 2007, where we presented the first-ever structure of the TRPV1 ion channel's ankyrin repeat domain, a key regulatory domain of multiple temperature-sensitive TRP channels.

a. Lishko P.V., Procko E., Jin X., Phelps C.B., Gaudet R. The ankyrin repeats of TRPV1 bind multiple ligands and modulate channel sensitivity. *Neuron.* 2007 Jun 21;54(6):905-18 (cited over 550 times)

3. **Expertise in electrophysiology and mitochondrial biology**. I developed and refined the patch-clamp technique for studying cellular organelles, such as the sperm flagellum and mitochondria, significantly advancing our understanding of sperm physiology and mitochondrial function. In mitochondria, my work uncovered the molecular mechanism of uncoupling via UCP1 (Cell, 2012). I conducted the first whole-cell patch-clamp recording of human sperm and identified Hv1 as its principal H+ channel (Cell, 2010). I also discovered the mechanism of sperm activation by progesterone, solving a 20-year mystery (Nature, 2011). As an independent investigator, I identified the key K+ channel in human sperm that regulates membrane potential and discovered TRPV4 as the temperature-sensitive sperm channel. Over the past 15 years, this technique has facilitated the identification of all major sperm ion channels and is now widely used by research labs worldwide.

a. 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 (Journal Cover, Paper Flick, Previews; cited over 420 times)
b. Lishko PV, Botchkina IL, Kirichok Y. *Nature* 2011; 471: 387-91. (Journal Cover, News & Views; cited over 740 times)
c. A Fedorenko, PV Lishko, Y Kirichok. Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. *Cell* 2012;151 (2), 400-413; PMID 23063128; (cited 1086 times)

4. **Expert in steroid signaling in the brain and eye epithelia, focusing on unconventional pathways where steroids activate ion channels via membrane receptors**. My team has identified ABHD2 as the first steroid-activated endocannabinoid hydrolase, revealing a novel steroid-endocannabinoid link regulating reproductive and epithelial tissue physiology. My team discovered that ABHD2, upon binding progesterone, breaks down 2-AG, an inhibitor of calcium channels, and this mechanism plays a role in ovarian aging and choroid plexus function. We also uncovered the Kir7.1 ion channel as a direct target of steroids, playing a key role in the choroid plexus and retinal pigment epithelium. Our current research focuses on the molecular mechanisms of Kir7.1's interaction with steroids and its role in age-related diseases. For years, my lab has developed biophysical, biochemical, and cell biology methods to study steroid regulation in tissues, with a recent focus on aging in the brain and sensory epithelia.

a. Miller MR, Mannowetz N, Iavarone AT, Safavi R, Gracheva EO, Smith JF, Hill RZ, Bautista DM, Kirichok Y, Lishko P.V. Unconventional endocannabinoid signaling governs sperm activation via sex hormone progesterone. *Science.* 2016 Apr 29;352(6285):555-9. doi: 10.1126/science. aad6887. PMID: 26989199 (cited over 250 times)
b. Björkgren I, Mendoza S, Chung DH, Haoui M, Petersen N, and Lishko PV. The epithelial potassium channel Kir7.1 is stimulated by progesterone.. *J of Gen Physiology.* 2021. Oct 4;153(10):e202112924. doi: 10.1085/jgp.202112924. Epub 2021 Aug 13. PMID: 34387656
c. Haoui M, Petersen NT, Björkgren I, Chung DH, and Lishko P.V. Choroid plexus epithelial cells as a model to study nongenomic steroid signaling and its effect on ion channel function. *Methods Enzymol*. 2021, 654:297-314. doi: 10.1016/bs.mie.2021.03.004. Epub 2021 Apr 7.PMID: 34120718

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

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BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Zhang, Rui

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

POSITION TITLE: Associate Professor of Biochemistry

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
Nanjing University, Nanjing, China	B.S.	06/2005	Biochemistry
Baylor College of Medicine, Houston, TX	Ph.D.	08/2010	Structural Biology
Lawrence Berkeley National Laboratory, Berkeley, CA	Postdoctoral	08/2015	Structural Biology

A. Personal Statement

My lab opened in December 2016. We primarily use high-resolution cryo-electron microscopy (cryo-EM) to study the structures and functions of microtubule-related macromolecular assemblies from native sources. I have 18 years' experience in cryo-EM, and I have a track record of developing novel data processing algorithms to tackle difficult biological samples, especially those with pseudo-symmetry. In 2019, using single particle cryo-EM, we determined the atomic structure of ciliary doublet microtubules (DMT) isolated from *Chlamydomonas*, and identified 38 ciliary microtubule associated proteins (1). Our work was chosen as the cover of a textbook (Molecular Cell Biology, 9th Edition, Lodish et al.). From the same sample, later on we also solved the atomic structures DMT-associated radial spokes and central apparatus. We have also applied the same technique to solve the *ex vivo* structure of the subpellicular microtubules from *Toxoplasma Gondii.*

PI's background: I was trained as a structural biologist with two great scientists in the cryo-EM field. As a graduate student in Dr. Wah Chiu's lab at Baylor College of Medicine, I determined cryo-EM structures of an alphavirus and Alzheimer's Aβ amyloid fibrils. During my postdoctoral training in Dr. Eva Nogales' lab at UC Berkeley and Lawrence Berkeley National Laboratory, I determined the first set of atomic-resolution cryo-EM structures of microtubules in different nucleotide states (2). Furthermore, I developed a novel microtubule data processing algorithm, which enabled structural studies of many microtubule associated proteins that we couldn't tackle previously, due to their small footprints on the microtubule platform. In December 2016, I joined Washington University in St. Louis (WUSTL) as a faculty member, since then we primarily use high-resolution single particle cryo-EM to pursue the native structures of macromolecular complexes that are directly isolated from various organisms, ranging from green algae to mammals (3). Most of our current projects in the lab are related to microtubules and cilia. In this proposal, I will draw on my experience in high-resolution single particle cryo-EM to obtain atomic resolution structures of human Kir7.1 in different functional states and bound to different small molecules.

Most relevant publications to the proposed research:
* denotes co-first author; # denotes co-corresponding author

1. Ma M, Stoyanova, M, Rademacher G, Dutcher, SK, Brown A#, **Zhang R#**. Structure of the decorated ciliary doublet microtubule. Cell. 2019 Oct 31;179(4):909-922. PMID: 31668805 PMCID: PMC6936269
2. **Zhang R**, Alushin GM, Brown A, Nogales E. Mechanistic Origin of Microtubule Dynamic Instability and its Modulation by EB Proteins. Cell (2015) 162 (4):849-59. PMID: 26234155; PMCID: PMC4537847

3. Wang X*, Fu Y*, Beatty WL, Ma M, Brown A, Sibley LD#, **Zhang R**#. Cryo-EM structure of cortical microtubules from human parasite Toxoplasma gondii identifies their microtubule inner proteins. Nature Communications (2021) 12, 3065–14. PMID: 34031406; PMCID: PMC8144581

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2015 - 2016 Research Specialist I, Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA

2016 - 2024 Assistant Professor, Department of Biochemistry and Molecular Biophysics, Washington University in St. Louis, School of Medicine, St. Louis, MO

2024 - Associate Professor, Department of Biochemistry and Molecular Biophysics, Washington University in St. Louis, School of Medicine, St. Louis, MO

Other Experience and Professional Memberships

2014 - Member, Biophysical Society

2016 - Member, American Society for Cell Biology

Honors and Awards

None

C. Contributions to Science

* denotes co-first author; # denotes co-corresponding author

1. Atomic structures of the native complexes within the cilia (doublet microtubule, radial spokes and central apparatus): The axoneme of most motile cilia consists of nine doublet MTs arranged in a circle around a central pair of singlet MTs, generating a 9+2 arrangement. A large T-shaped protein complex named radial spoke connects the doublet MTs to the central apparatus. In our recent work (1-3), we used single-particle cryo-EM to visualize and build atomic models of the repeating structures of several native axonemal complexes (doublet microtubule, radial spoke and central apparatus), which reveals the identities, positions, repeat lengths, and interactions of more than 120 associated proteins including microtubule inner proteins (MIPs). These structures demonstrate how these proteins establish the unique architecture of doublet microtubules and maintain coherent periodicities along the axoneme. Our work provides a molecular atlas for interpreting genetic, biochemical, and physiological data from different cell types and for understanding the etiology of human ciliopathies.

1. Ma M, Stoyanova, M, Rademacher G, Dutcher, SK, Brown A#, **Zhang R**#. Structure of the decorated ciliary doublet microtubule. Cell (2019) 179(4):909-12. PMID: 31668805; PMCID: PMC6936269
2. Gui M, Ma M, Sze-Tu E, Wang X, Koh F, Zhong ED, Berger B, Davis JH, Dutcher SK, **Zhang R**#, Brown A#. Structures of radial spokes and associated complexes important for ciliary motility. Nat Struct Mol Biol (2021) 28, 29–37. PMID: 33318703; PMCID: PMC7855293
3. Gui M, Wang X, Dutcher SK, Brown A#, **Zhang R**#. Ciliary central apparatus structure reveals mechanisms of microtubule patterning. Nat Struct Mol Biol (2022), 29, 483–492. PMID: 35578023

2. Atomic structure of the subpellicular microtubules from human parasite *Toxoplasma Gondii*: Unlike the dynamic MTs found in the cytoplasm of metazoan cells, the specialized cortical MTs from *Toxoplasma gondii*, a prevalent human pathogen, are extraordinarily stable and resistant to detergent and cold treatments. Using single-particle cryo-EM, we determine their *ex vivo* structure and identify three proteins (TrxL1, TrxL2 and SPM1) as *bona fide* microtubule inner proteins (MIPs) (4). These three MIPs form a mesh on the luminal surface and simultaneously stabilize the tubulin lattice in both longitudinal and lateral directions. Consistent with previous observations, deletion of the identified MIPs compromises MT stability and integrity under challenges by chemical treatments. Our results provide the structural basis to understand the stability of cortical MTs and suggest an evolutionarily conserved mechanism of MT stabilization from the inside.

4. Wang X*, Fu Y*, Beatty WL, Ma M, Brown A, Sibley LD#, **Zhang R**#. Cryo-EM structure of cortical microtubules from human parasite Toxoplasma gondii identifies their microtubule inner proteins. Nature Communications (2021) 12, 3065–14. PMID: 34031406; PMCID: PMC8144581

3. Mechanistic understanding of the microtubule dynamics and its regulation by associated proteins: During my postdoctoral training at Dr. Eva Nogales' lab, I used single-particle cryo-EM to determine the high-resolution structures of microtubules in different nucleotide states, and in complex with a number of microtubule associated proteins (MAPs) (5). On the technical side, I developed a novel microtubule data processing algorithm, which can accurately determine α,β-tubulin register and microtubule seam location without the need for additional protein markers for tubulin heterodimer (6). This protocol enabled structural studies of many microtubule associated proteins that we couldn't tackle previously, due to their small footprints on the microtubule platform. Our work shed light on the mechanistic origin of MT dynamic instability governed by GTP hydrolysis (5), and its modulation by EBs (7), an important family of proteins that track the plus-ends of microtubules; and by TPX2, another important protein that play multiple functions during the mitotic spindle assembly process (8).

5. **Zhang R**, Alushin GM, Brown A, Nogales E. Mechanistic Origin of Microtubule Dynamic Instability and its Modulation by EB Proteins. Cell (2015) 162(4):849-59. PMID: 26234155; PMCID: PMC4537847
6. **Zhang R**#, Nogales E#. A New Protocol to Accurately Determine Microtubule Lattice Seam Location. Journal of Structural Biology (2015) 192(2):245-54. PMID: 26424086; PMCID: PMC4634897
7. **Zhang R**#, LaFrance B, Nogales E#. Separating the effects of nucleotide and EB binding on microtubule structure. Proc Natl Acad Sci USA (2018) PMID: 29915050; PMCID: PMC6142192.
8. **Zhang R***, Roostalu J*, Surrey T#, Nogales E#. Structural insight into TPX2-stimulated microtubule assembly. Elife. (2017) Nov 9;6. doi: 10.7554/eLife.30959. PMID: 29120325; PMCID: PMC5679754

4. Cryo-EM structures of other macromolecular assemblies: During my graduate and postdoctoral researches, I used cryo-EM to study a number of proteins or protein complexes with various symmetries. Briefly, I have solved the cryo-EM structure of the Alzheimer's Aβ amyloid fibrils with helical symmetry (9). I have also obtained the first near-atomic resolution cryo-EM structure of an enveloped alphavirus that infect humans (10). More recently, we have used cryo-EM to elucidate how the small GTPase Arf1 activates AP complexes for cargo binding (11).

9. **Zhang R**, Hu X, Khant H, Ludtke SJ, Chiu W, Schmid MF, Frieden C, Lee JM. Interprotofilament interactions between Alzheimer's Aβ1-42 peptides in amyloid fibrils revealed by cryoEM. Proceedings of the National Academy of Sciences (2009) 106(12):4653-58. PMID: 19264960; PMCID: PMC266077
10. **Zhang R**, Hryc CF, Cong Y, Liu X, Jakana J, Gorchakov R, Baker ML, Weaver SC, Chiu W. 4.4 Å cryo-EM structure of an enveloped alphavirus Venezuelan equine encephalitis virus. EMBO Journal (2011) 30(18):3854-63. PMID: 21829169; PMCID: PMC3173789
11. Shen QT*, Ren X*, **Zhang R***, Lee IH, Hurley JH. HIV-1 Nef hijacks clathrin coats by stabilizing AP-1:Arf1 polygons. Science (2015) 350(6259):aac5137. PMID: 26494761; PMCID: PMC4638387

Complete List of Published Work in MyBibliography:

I have published 28 peer-reviewed articles. Of these, I have been the (co-)first or corresponding author (sole or joint) on 15 of them. This list includes 2 review articles. For a complete list of my published works, please see:

https://www.ncbi.nlm.nih.gov/sites/myncbi/1X_CdlpZNlVQ5/bibliography/40604497/public/?sortby=pubDate&sdirection=descending

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: Qingwei Niu

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

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)*	Completion Date MM/YYYY	FIELD OF STUDY
Rutgers University-New Brunswick	BA	05/2018	Biological Science
Johns Hopkins Bloomberg School of Public Health	MS	05/2021	Molecular Microbiology and Immunology
Washington University of St. Louis	PHD	05/2026 (Expected)	Molecular Cell Biology

A. Personal Statement

My first scientific research started during my junior year at Rutgers University. I worked as research assistant to Dr. Ilya Raskin, exploring the molecular effects of plant-derived compounds in the quest to develop potential botanical anti-inflammatory therapeutics. The results were published on PloS One (Skubel et al. 2018) and presented in my senior honor thesis defense (George H. Cook Scholars Program). My first achievement in developing the RAMES technique allowed me to experience how progress in research could start with something small that might grow to have global ramifications. This gave me more confidence to become a scientific researcher. To build up a solid foundation for a Ph.D. program, I started my master's study at Johns Hopkins Bloomberg School of Public Health. I joined Dr. J. Marie Hardwick's lab to cultivate my knowledge in cellular and molecular fields. With the master's education, I have a deeper and more comprehensive understanding on being a qualified academic researcher. I learned the value of carefully analyzing every experiment result, even it is negative data. These habits always allow me to generate new ideas for my project, which honed my logical thinking ability and rapidly improved my experimental skills. My prior experiences have informed my current research goal, which is improving people's health from pathogenesis perspective to discover potential therapeutic targets of various human diseases. To achieve my research goal, I entered the fields of physiology and biochemistry during my graduate training at Washington University, where I study the potassium channel regulated phagocytosis in RPE cells under the mentorship of Dr. Polina Lishko, a renowned scientist known for her pioneering work in the field of electrophysiology, and Dr. Rui Zhang. With the recently adopted techniques of patch clamp and cryo-electron microscopy, I am exploring the role of Kir7.1 channel in RPE phagocytosis function through structural and electrophysiological perspectives. The academic education and research exposure I've received thus far have furnished me with a strong foundation in the fields of molecular biology and biochemistry. To translate my knowledge to fully help others, I also worked at the emergency department in Johns Hopkins Hospital by holding consultations with patients and families to propagate HIV/HCV knowledge and perform rapid HIV/HCV tests on patients. I also trained and mentored a summer undergraduate researcher in several techniques such as plasmids purification, western blot, mouse eye dissection, primary and immobilized cell culture. Overall, I believe that the mentors I have chosen, the research career I have pursued, and the guidance I have received through the grant will provide a solid foundation for my ultimate aspiration of becoming a scholar in academia.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022 – Present	Pre Doc trainee, Washington University in St. Louis, Dr. Polina Lishko's laboratory
2022 – Present	Pre Doc trainee, Washington University in St. Louis, Dr. Rui Zhang's laboratory
2020 – 2021	Graduate Research Assistant (MS), Johns Hopkins University, Dr. Fidel Zavala's laboratory
2020 – 2021	Graduate Research Assistant (MS), Johns Hopkins University, Dr. J. Marie Hardwick's laboratory
2020 – 2021	Teaching Assistant, Johns Hopkins University, Immunology, Infection and Disease
2019.10 – 2019.12	Counselor, Johns Hopkins Hospital--Emergency Department, Generation Tomorrow-the HIV & HCV Training Program, Johns Hopkins Center for AIDS Research
2017 – 2018	Volunteer, Saint Peter's University Hospital
2017 – 2018	Undergraduate Researcher, Rutgers University, Dr. Ilya Raskin's laboratory

Honors

2020	Master's Tuition Scholarship, Johns Hopkins University, MD
2018	George H. Cook Scholar, Rutgers University, NJ

C. Contributions to Science

1. **Undergraduate Research:** My project tackled one major challenge limiting botanical research caused by degradation of plant bioactive metabolomes during the transport from collection fields to labs. This problem incurred huge wastes on time, labor, and research funding. Therefore, I worked to stabilize plants active metabolites by developing a new extraction method through simplifying the time-consuming operation, adjusting procedures fitting for single person processing, and increasing conservation time of plant extractions with limited decay. The ultimate technique, which named as RAMES, allows botanical extracts to be stored within filter disks at -20^{o}C for 12 months. Utilizing RAMES, I was able to screen various plant tissue extracts collected in South Africa, as well as spices from India, China, and Thailand to identify natural anti-inflammatory compounds.
    a. Skubel, S., Dushenkov, V., Graf, B., **Niu, Q.**, Poulev, A., Kalariya, H., Foxcroft, L., Raskin, I. [2018], Rapid, field-deployable method for collecting and preserving plant metabolome for biochemical and functional characterization. PLoS One, Vol. 13, No.9, article e0203569.
    b. **Niu, Q.** "Further Development of Screens-To-Nature Methods in Relationship to Plant Extracts" Undergraduate Thesis Defense (Submitted to Honors Committee, the George H. Cook Scholars Program). New Brunswick, New Jersey, April 12, 2018.

2. **Graduate Research (MS):** Lysosomes as key compartments in cells are associated with various cellular activities, including nutrient sensing and Ca2+ signaling. One of the crucial complexes active at lysosome is mTORC1. I was very fortunate to study a novel signaling pathway that has important influence on lysosomal acidification process and regulating autophagy. The main goal of my project focused is to explore the mechanism of a novel mTORC1 regulator by identifying interacting proteins based on our lab's work in yeast, utilizing both in vivo (mice) and in vitro (HeLa cell lines) methodologies. This project allows the identification of a novel mammalian nutrient-sensing pathway suppressing mTORC1, which could be a novel therapeutic target to inhibit mTORC1 activity and lead to the anti-cancer effect. Yeast Whi2 were proved to interact with phosphatases Psr1 and Psr2 to inhibit TORC1 kinase activity under low amino acid conditions. My project was initiated after discovering that the mammalian equivalent of Whi2, KCTD proteins, had a conserved function to regulate mammalian TORC1 (mTORC1) activity, with a family of proteins (C-terminal domain-containing phosphatases or CTDSPs) which were functionally similar to yeast phosphatases Psr1 and Psr2. Various cellular and molecular assays are performed to understand the biological function of KCTDs and CTDSPs, such as examining the activity of mTORC1 under various CTDSP overexpression or under nutrient starvation assays in HeLa cell extract. Biochemical methods, like co-immunoprecipitation (co-IP) assays, are also used to have a more comprehensive understanding of KCTDs and CTDSPs from their structural level. A major challenge is the weak interaction between target proteins. I adjusted co-IP assays through various perspectives, including adjusting plasmids construction, reducing transfection toxicity, testing different types of pull-down beads, and increasing washing volume to decrease the non-specific binding. Ultimately, I was able

to generate a solid reproducible protein interaction result with optimized co-IP method. Additionally, I have participated in an in vivo study, utilizing mice hippocampus dissection to study the mTORC1 activity under various target proteins knockout conditions. Finally, based on language advantage, I helped in screening various KCTD7 mutations in Chinese patients to uncover potential connections between patient KCTD7 mutations and their epilepsy symptoms.

a. **Niu, Q.** "Do mammalian cells have the novel Whi2-Psr1/2 mTORC1 signaling pathway recently identified in yeast?" Departmental Research Forum. Baltimore, Maryland, March 23, 2020.
b. **Niu, Q.** "Biochemical Interactions of the Human Epilepsy Protein KCTD7" Master Thesis Defense. Baltimore, Maryland, May 18, 2021.

3. **Graduate Research (PHD):** Age-related macular degeneration (AMD) is the primary cause of blindness among people over 55 years of age and is characterized by retinal pigment epithelium (RPE) degeneration. KCNJ13 gene encodes polypeptide that forms a tetramer, which in its turn acting as inwardly rectifying potassium ion channel Kir7.1. This potassium ion channel locates at the apical aspects of the RPE. It is vital for normal RPE function to maintain ion homeostasis, as well as to support and nurture retina photoreceptors. Dysfunctional Kir7.1 channels is associated with early onset of blindness such as Snowflake Vitreoretinal Degeneration (SVD) and Lebers Congenital Amaurosis (LCA16) in humans, mainly due to dysfunctional RPE. Intriguingly, progesterone was confirmed as a natural positive regulator of Kir7.1 channel on RPE cells in electrophysiology studies. My work focus on understanding the effect steroids impose on age-related changes in the RPE through investigating the cryo-EM structure of the human Kir7.1 channel conformation, developing high-throughput screen model to test activators of Kir7.1, and exploring the regulation mechanism of this channel on RPE phagocytosis function. Structural biology skills on how to purify membrane proteins via immobilized metal affinity chromatography and gel filtration with ÄKTA™ pure to unmask the 3D structure of Kir 7.1 under cryo-EM will be adopted in collaborator's lab. This boosts up my accumulation on biochemistry, allowing me to link the Kir7.1 studied protein function with its 3D structure. I believe my proficient skills in cell culture, protein purification, co-Immunoprecipitation, immunostaining, immunoblotting, gel electrophoresis will largely support my study on Kir7.1 channel.