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: Wu, Kathryn

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

POSITION TITLE: Graduate Student

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	START DATE (MM/YYYY)	END DATE (MM/YYYY)	FIELD OF STUDY
Swarthmore College	BA	08/2010	05/2014	Biology
Stanford University, School of Medicine	PHD	08/2016	06/2024	Medicine
Stanford University, School of Medicine	MD	08/2016	06/2024	Neuroscience

A. Personal Statement

When I was in college, a neurological disease known as amyotrophic lateral sclerosis (ALS) progressively robbed my mom of her movement, voice, and autonomy, claiming her life just 10 months after diagnosis. Witnessing both the value of medicine and its limitations propelled me to pursue a career as a physician scientist. My goal is to uncover therapeutically-relevant molecular mechanisms of ALS and other neurological diseases, and I have sought out educational and training opportunities accordingly.

Prior to graduate school, I investigated the pathophysiology of ALS for 3 years in 2 different laboratories. As an undergraduate at Swarthmore, I worked with Edward Lee, MD, PhD, at the University of Pennsylvania to study promoter methylation of an ALS gene. This experience equipped me to perform molecular biology experiments, think critically, and independently revise my initial hypotheses in light of my data. My work in Dr. Lee's lab formed the basis of my undergraduate honors thesis, an oral presentation at the Leadership Alliance National Symposium, and co-authorship on 2 publications. Following graduation, I worked with Robert Baloh, MD, PhD, to improve ALS modeling by genetically editing patient-derived induced pluripotent stem cells (iPSCs) to generate isogenic control lines. Here I expanded my molecular biology toolset, mastered cell culture techniques, gained troubleshooting experience, and thought deeply about various strategies for modeling neurological diseases. I presented my work as a poster at the California ALS Research Summit and contributed to two publications.

I am currently pursuing an MD-PhD at Stanford University. In the process of choosing my PhD lab, I rotated through an ALS lab and contributed to a publication on the normal function of an ALS gene. However, I ultimately decided to venture outside of direct ALS research because I want to acquire fresh perspectives and techniques to bring back into the field. For now, I have chosen to focus on the processes that promote the normal development and health of the nervous system rather than the ones that contribute to aging and disease. This has led me to develop a fascination with glia, a class of cells which keep neurons alive and functioning. I am particularly captivated by oligodendrocytes, which spirally wrap their processes around axons and compact the membranes within these processes to establish insulatory layers of myelin. I am currently working with my sponsor, Brad Zuchero, PhD, and cosponsor, Wah Chiu, PhD, to understand the molecular mechanisms that drive myelin wrapping and compaction. Dr. Zuchero made landmark discoveries about the role of the actin cytoskeleton during myelination, while Dr. Chiu is a leading expert on cryo-electron microscopy. With the combined guidance of Dr. Zuchero and Dr. Chiu, I am well-positioned to surmount current technological hurdles in the myelin field to glean novel mechanistic insights about how oligodendrocytes myelinate axons.

Positions and Employment

2013	Teaching Assistant, Introductory Biology, Swarthmore College
2013	Head Biology Writing and Speaking Tutor, Writing Center, Swarthmore College
2013 - 2014	Undergraduate researcher, Edward Lee Lab, University of Pennsylvania
2014 - 2016	Research Associate, Robert Baloh Lab, Cedars-Sinai Medical Center

2017	Teaching Assistant, The Nervous System, Stanford University School of Medicine
2017 - Present	Graduate Student, Zuchero Lab, Stanford University School of Medicine

Other Experience and Professional Memberships

2011 - Present	Member, Sigma Xi
2014 - Present	Member, Phi Beta Kappa
2016 - Present	Member, American Medical Association
2018 - Present	Member, Society for Neuroscience
2018 - Present	Member, Stanford Women Association of Physician Scientists

Honors

2011	Robert Enders Field Biology Award, Swarthmore College
2013 - 2014	Evenor Armington Scholarship, Swarthmore College
2013 - 2014	Edward Martin Scholarship, Swarthmore College
2014	Honors, Swarthmore College
2014	Leva Memorial Prize, Swarthmore College
2016 - Present	T32 Institutional National Research Service Award, NIH
2020	Finalist, Paul and Daisy Soros Fellowship for New Americans
2020	Stanford Interdisciplinary Graduate Fellowship

Standardized Test Scores

2015	MCAT: 521 (99^{th} percentile)
2018	USMLE Step 1: 247 (80^{th} percentile)

C. Contribution to Science

1. **ALS and FTD pathophysiology**. *Undergraduate research, laboratory of Dr. Edward Lee*

The most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) is a repeat expansion mutation in the *C9ORF72* (C9) gene. Patients with the C9 expansion (C9 carriers) were reported to have reduced C9 expression, but how expression is reduced and whether it contributes to disease was unknown. Under Dr. Edward Lee's guidance, I tested cells and post-mortem tissue to determine whether the C9 promotor is more methylated in C9 carriers, as this could be a mechanism of transcript reduction. I found that the C9 promoter was indeed more methylated in C9 carriers, and that increased promotor methylation tightly correlates with decreased C9 expression. I also correlated C9 promoter methylation with clinical measures in a small cohort of patients and found that FTD patients with increased promotor methylation lived longer. This led me to independently hypothesize that promoter methylation could be protective by suppressing the expression of potentially toxic expansion-associated RNA and protein products. Other lab members went on to show that C9 promoter methylation is indeed associated with fewer of these RNA and protein products, as well as slower clinical decline in a larger cohort of patients. This research has implications for the development of therapeutics for C9-associated disease and, to our knowledge, represents the first instance in which a mutation-associated epigenetic modification has been suggested to be protective in neurodegeneration. I co-authored two papers on this topic and gave an oral presentation on my work at the National Leadership Alliance Symposium.

a. Liu EY, Russ J, **Wu K**, Neal D, Suh E, McNally AG, Irwin DJ, Van Deerlin VM, Lee EB. C9orf72 hypermethylation protects against repeat expansion-associated pathology in ALS/FTD. Acta Neuropathol. 2014 Oct;128(4):525-41. PubMed PMID: 24806409; PubMed Central PMCID: PMC4161616.
b. Russ J, Liu EY, **Wu K**, Neal D, Suh E, Irwin DJ, McMillan CT, Harms MB, Cairns NJ, Wood EM, Xie SX, Elman L, McCluskey L, Grossman M, Van Deerlin VM, Lee EB. Hypermethylation of repeat expanded C9orf72 is a clinical and molecular disease modifier. Acta Neuropathol. 2015 Jan;129(1):39-52. PubMed PMID: 25388784; PubMed Central PMCID: PMC4282973.
c. [Undergraduate Honors Thesis] **Wu K**, Liu EY, Russ J, Lee EB. Promoter hypermethylation of expanded C9ORF72 may play a protective role in ALS and FTD patients. 2014 May.

d. [Oral Presentation] **Wu K**, Liu EY, Russ J, Lee EB. Increased hypermethylation of mutant *C9ORF72* promoter in affected brain regions of ALS and FTD patients. Leadership Alliance National Symposium; 2013 Jul 26-28; Stamford, CT.

2. ALS modeling. Post-baccalaureate research, laboratory of Dr. Robert Baloh

Patient-derived induced pluripotent stem cell (iPSC) lines have been used to model the most common genetic form of ALS, which is linked to a repeat expansion mutation in the *C9ORF72* (C9) gene. However, because of the inherent genetic variability between individuals, it is difficult to extract disease-relevant insights from comparisons between C9-ALS and control cells. To avoid this problem, I used the CRISPR-Cas9 genome-editing system to generate isogenic control lines, or cells which lack the C9 expansion but are otherwise genetically identical to the ALS cells. I confirmed that these isogenic control lines have normal karyotypes, differentiate normally into motor neurons, and lack the expansion-associated RNA and protein products found in C9-ALS motor neurons. Interestingly, I found C9-ALS motor neurons have reduced C9 RNA transcripts compared to their isogenic controls, suggesting a causal link between the expansion and reduced C9 expression. I began this project working closely with Dr. Kim, a postdoctoral scholar in the lab, but became increasingly independent in executing and troubleshooting experiments. I presented this work as a poster at the California ALS Research Summit, and the isogenic control lines are now widely available through the Cedars Sinai iPSC Core Repository. Other researchers at Cedars Sinai performed a single-cell transcriptomics study on C9-ALS and isogenic control motor neurons, and I am the 5th author on a manuscript in preparation. In addition to working on cellular models of ALS, I helped Dr. O'Rourke in my lab characterize a novel C9ORF72 mutant mouse and am a middle co-author on the resulting paper.

a. [poster] Kim, K.*, **Wu, K.***, Muhammad, A.K.M., Simpkinson, M., Harms, M., and R.H. Baloh (*These authors contributed equally). Improving ALS-in-a-Dish modeling by using CRISPR/Cas9 to generate isogenic control lines for *C9ORF72* patient-derived iPSCs. California ALS Research Summit; 2016 Jan 10-11; San Diego, CA.
b. Ho R, Workman MJ, Mathkar P, **Wu K**, Kim KJ, O'Rourke JG, Kellogg M, Montel V, Banuelos MG, Arogundade OA, Diaz-Garcia S, Oheb D, Huang S, Khrebtukova I, Watson L, Ravits J, Taylor K, Baloh RH, Svendsen CN. Cross-Comparison of Human iPSC Motor Neuron Models of Familial and Sporadic ALS Reveals Early and Convergent Transcriptomic Disease Signatures. Cell Syst. 2021 Feb 17;12(2):159-175.e9. doi: 10.1016/j.cels.2020.10.010. Epub 2020 Dec 30. PMID: 33382996; PMCID: PMC7897311.
c. Isogenic control ALS iPSC lines available through the Cedars-Sinai iPSC Core Repository: https://biomanufacturing.cedars-sinai.org/
d. O'Rourke JG, Bogdanik L, Muhammad AKMG, Gendron TF, Kim KJ, Austin A, Cady J, Liu EY, Zarrow J, Grant S, Ho R, Bell S, Carmona S, Simpkinson M, Lall D**, Wu K**, Daughrity L, Dickson DW, Harms MB, Petrucelli L, Lee EB, Lutz CM, Baloh RH. C9orf72 BAC Transgenic Mice Display Typical Pathologic Features of ALS/FTD. Neuron. 2015 Dec 2;88(5):892-901. PubMed PMID: 26637796; PubMed Central PMCID: PMC4672384.

3. Myelination and radial sorting. *Dissertation research, Dr. Brad Zuchero*

My graduate research in the Zuchero lab centers on the cellular and molecular mechanisms of myelination. My primary project focuses on the role of actin disassembly on myelin compaction, which is critical for establishing the insulating properties of myelin. Myelin compaction describes the process by which oligodendrocytes seal together their membranes, squeezing out the cytoplasm in-between. I used bio-AFM to discover that cultured primary rat oligodendrocytes, which extend large, flat membrane sheaths, compact their membranes *in vitro*. I developed a fluorescence-based assay for compaction and used live-imaging to study spatiotemporal dynamics of compaction. I then tested the effects of pharmacological and genetic actin-modulators on compaction and found that disassembling actin promotes compaction. I performed cryo-tomography on cultured oligodendrocytes and am currently processing the data to determine whether actin filaments are absent from compact regions. I am further working towards performing cryo-tomography on mouse spinal cord to determine whether actin filaments are absent in the myelinating processes of oligodendrocytes *in vivo*. Altogether, my data support a model in which actin disassembly serves as a prerequisite step for myelin compaction.

a. Lam M, Takeo K, Almeida RG, Cooper MH, **Wu K**, Iyer M, Kantarci H, Zuchero JB. CNS myelination requires VAMP2/3-mediated membrane expansion in oligodendrocytes. PubMed PMID: 36151203. PMCID: PMC9508103.
b. Chai N, Haney M, Couthouis J, Morgens D, Benjamin A, **Wu K**, Ousey J, Fang S, Finer S, Bassik M, Gitler A. Genome-wide synthetic lethal CRISPR screen identifies FIS1 as a genetic interactor of ALS-linked C9ORF72. Brain Res. 2020 Feb 1;1728:146601. PubMed PMID: 31843624.

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: Wu, Gong-Her

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

POSITION TITLE: Research Scientist

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
National Chung-Hsing University, Taichung, Taiwan	BS	07/2004	Life Sciences
National Tsing-Hua University, Hsinchu, Taiwan	MS	07/2006	Molecular and Cellular Biology
National Tsing-Hua University, Hsinchu, Taiwan	PHD	07/2015	Molecular and Cellular Biology
Academia Sinica, Taipei, Taiwan	Postdoctoral Fellow	10/2016	Nanoparticle, X-ray microscope tomography
Baylor College of Medicine, Houston, TX	Postdoctoral Fellow	06/2017	Cryogenic electron microscopy
Stanford University, Stanford, CA	Postdoctoral Fellow	08/2021	Huntington's disease, Cryogenic electron tomography (cryo-ET)
Stanford University, Stanford, CA	Research Scientist	To present	Huntington's disease, neuronal disease, mitochondria, virology, Cryo-Ligh Electron Microscopy (CLEM), Cryo-Focused ion beam (cryo-FIB) and Cryogenic electron tomography (cryo-ET)

A. Personal Statement

My research interests focus on using cryogenic electron tomography (cryo-ET) to investigate disease-related neuronal structure disorders. My career goal is to become an independent principal investigator in an academic institution and continue to investigate the cause and potential treatment strategies for human neuronal diseases.

I have extensive research experience in molecular and neuronal biology, especially in mitochondria structure and protein aggregations. My Ph.D. dissertation focused on kinase-mediated anterograde motor protein motility and cluster status changes on neuron cells' axonal microtubules. After I received my Ph.D. degree, I joined Dr. Yeu-Kuang Hwu's laboratory in Academia Sinica (Taiwan) as a postdoctoral scholar to investigate neuronal networks in the brain tissues and primary cultured brain cells using hard X-ray microscopy and 3D image reconstruction. These experiences equipped me with the knowledge and skills to study protein structures of diseased neurons in mammalian brain tissue.

I then decided to pursue cryo-ET systems for investigating neuron proteins in their native, in vivo status. Therefore, I came to the U.S. and joined Dr. Wah Chiu's laboratory at Baylor College of Medicine and Stanford University. My research project focuses on studying mutated Hungtinting (mHTT) protein aggregation structures in Huntington's disease (HD) neurons by cryo-ET. To optimize and orchestrate multiple microscopy systems for precisely locating protein in the cells with cryo-ET, I established an operation procedure for multi-scaled 3D Cryo-EM imaging and published this novel method in *Structure* (2020). Furthermore, my colleague and I combined cryo-ET and proteomic bioinformatic analysis with artificial intelligence-based automated annotation to interrogate the early disease state biomarker: mitochondrial RNA granules and double membrane-

bound sheet-like aggregates in Huntington's disease induced pluripotent stem cell (iPSC)-derived neuron (*Nat Commun*, 2023). My current study focuses on unveiling HD disease causes and its biomarkers by investigating the novel structures I identified from mHTT aggregations and their correlation to altered organelles structures in tissue levels such as HD brain organoid and HD mouse brain. In addition, I am also working on projects related to the Covid-19 drug discovery and calcium calcification of mitochondria in optic disc drusen.

My long-term career goal is to become an independent scientific researcher who contributes to understanding pathological biological phenomena of neuronal, mitochondria, and virus disease by cryo light electron microscopy (CLEM), cryo-focus ion beam (cryo-FIB) and cryo-ET.

1. **Wu GH**, Smith-Geater C, Galaz-Montoya JG, Gu Y, Gupte SR, Aviner R, Mitchell PG, Hsu J, Miramontes R, Wang KQ, Geller NR, Hou C, Danita C, Joubert LM, Schmid MF, Yeung S, Frydman J, Mobley W, Wu C, Thompson LM, Chiu W. CryoET reveals organelle phenotypes in huntington disease patient iPSC-derived and mouse primary neurons. Nat Commun. 2023 Feb 8;14(1):692. PMID: 36754966; PMCID: PMC9908936.

2. **Wu GH**, Mitchell PG, Galaz-Montoya JG, Hecksel CW, Sontag EM, Gangadharan V, Marshman J, Mankus D, Bisher ME, Lytton-Jean AKR, Frydman J, Czymmek K, Chiu W. Multi-scale 3D Cryo-Correlative Microscopy for Vitrified Cells. Structure. 2020 Aug 15; PubMed PMID: 32814034.

3. **Wu GH**, Muthaiyan Shanmugam M, Bhan P, Huang YH, Wagner OI. Identification and Characterization of LIN-2(CASK) as a Regulator of Kinesin-3 UNC-104(KIF1A) Motility and Clustering in Neurons. Traffic. 2016 Aug;17(8):891-907. PubMed PMID: 27172328.

B. Positions and Honors

Positions and Employment

2007 - 2015	Graduate Research Assistant, National Tsing-Hua University, Hsinchu
2015 - 2016	Postdoctoral Fellow, Institute of Physics, Academia Sinica, Taipei
2016 - 2017	Postdoctoral Associates, National Center for Macro-molecular Imaging, Baylor College of Medicine, Houston, TX
2017 - 2022	Postdoctoral Scholar, Department of Bio-engineering, Stanford University, Stanford, CA
2022 - present	Research Scientist, Department of Bio-engineering, Stanford University, Stanford, CA

Other Experience and Professional Memberships

2019 -	member, American Association for the Advancement of Science

Honors

2006 - 2007	Out-standing Ph.D. Student Scholarship, National Tsing-Hua University, Taiwan
2011 - 2011	Student Travel Award, National Tsing-Hua University, Taiwan
2012 - 2012	Excellent Award, Student Poster Competition of Life Science, National Tsing-Hua University, Taiwan
2017 - 2018	Recipient of Ministry of Science and Technology Overseas Project for Post Graduate Research, Ministry of Science and Technology, Taiwan
2019 - 2023	Hereditary Disease Foundation Post-Doctoral Fellowship, Hereditary Disease Foundation, USA

C. Contribution to Science

1. Regulation of motor protein kinesin-3 KIF1A/UNC-104 motility and clustering in neurons.

In this project, I focused on the mechanisms of protein kinase LIN-2 mediated kinesin motor protein UNC-104 activity regulation in *C. elegans.* I created LIN-2 mutants for UNC-104 interaction domain mapping using bimolecular fluorescence complementation assays. I discovered that the absence of the motor-activating function of LIN-2 results in increased motor clustering along axons by using fluorescence confocal microscopy,

thus retaining cargos in neuron cell bodies. I also contributed in projects that focused on UNC-104 regulations mediated by different kinesin adaptor proteins such as Tau and SYD-2. These studies reveal that Tau and SYD-2 are also essential for UNC-104 motility regulations in neuronal cells.

a. **Wu GH**, Muthaiyan Shanmugam M, Bhan P, Huang YH, Wagner OI. Identification and Characterization of LIN-2(CASK) as a Regulator of Kinesin-3 UNC-104(KIF1A) Motility and Clustering in Neurons. Traffic. 2016 Aug;17(8):891-907. PubMed PMID: 27172328.

b. Tien NW, **Wu GH**, Hsu CC, Chang CY, Wagner OI. Tau/PTL-1 associates with kinesin-3 KIF1A/UNC-104 and affects the motor's motility characteristics in C. elegans neurons. Neurobiol Dis. 2011 Aug;43(2):495-506. PubMed PMID: 21569846.

c. Wagner OI, Esposito A, Köhler B, Chen CW, Shen CP, **Wu GH**, Butkevich E, Mandalapu S, Wenzel D, Wouters FS, Klopfenstein DR. Synaptic scaffolding protein SYD-2 clusters and activates kinesin-3 UNC-104 in C. elegans. Proc Natl Acad Sci U S A. 2009 Nov 17;106(46):19605-10. PubMed PMID: 19880746; PubMed Central PMCID: PMC2780759.

2. Negative effects of nanoparticles in neuronal development.

During my Ph.D. training, I initiated and actively involved in collaboration projects with Dr. Ta-Jen Yen's lab in Department of Materials Science and Engineering, National Tsing Hua University. We used *C. elegans* as model organism to investigate the toxicity of titanium dioxide (TiO2) nanoparticles in neuron development. Our results revel that TiO2 will be uptake by neuron, and results in decreasing axonal growth and thus impedes locomotion behavior of *C. elegans*. Furthermore, we confirmed this development interruption is due to abnormal gene expression by using DNA array chip. Beside TiO2, we also found nano gold particles have toxicity effects in *C. elegans* development. These studies indicates that using nanoparticles in imaging neuronal cells may artificially interfere biological functions and behaviors of neurons.

a. Hu CC, **Wu GH**, Lai SF, Muthaiyan Shanmugam M, Hwu Y, Wagner OI, Yen TJ. Toxic Effects of Size-tunable Gold Nanoparticles on Caenorhabditis elegans Development and Gene Regulation. Sci Rep. 2018 Oct 15;8(1):15245. PubMed PMID: 30323250; PubMed Central PMCID: PMC6189128.

b. Hu CC, **Wu GH**, Hua TE, Wagner OI, Yen TJ. Uptake of TiO_2 Nanoparticles into C. elegans Neurons Negatively Affects Axonal Growth and Worm Locomotion Behavior. ACS Appl Mater Interfaces. 2018 Mar 14;10(10):8485-8495. PubMed PMID: 29464946.

3. Application of Cryo-EM in investigating structure and sub-cellar locations of Hungtintin protein aggregation.

I found a novel structure and its unique spatial distribution pattern of mutated Huntingtin (mHTT) protein in neuronal cells developed from HD patients' induced pluripotent stem cells (iPSC). I also found aberrant mitochondria structures in these disease neurons but not in iPSC neurons derived from healthy donors. I used cryo-ET, proteomic bioinformatic analysis, and artificial intelligence-based automated annotation to identify early state biomarkers: mitochondrial RNA granules and double membrane-bound sheet-like aggregates in Huntington's disease iPSC-derived neurons in *Nat Commun* (2023). In order to observe the HD cellular structure in the cell body, I established a workflow orchestrating cryo-confocal microscope, cryo-focus ion beam-scanning electron microscope, and cryo-transmission electron microscope together to identify the precise location of subject proteins in the cell. This workflow dramatically improves the successful rate of imaging rare proteins in precious patient samples. We published this novel imaging procedure in *Structure* (2020).

I currently focus on developing the tissue level cryo-FIB/SEM and cryo-ET workflow for HD disease biomarkers discovery in HD brain organoid and R6/1 (HD) mouse brain. Furthermore, my cooperators and I found drug candidates to rescue the mouse HD brain tissue disorder and mitochondria phenomena (unpublished data).

a. Wu GH, Mitchell PG, Galaz-Montoya JG, Hecksel CW, Sontag EM, Gangadharan V, Marshman J, Mankus D, Bisher ME, Lytton-Jean AKR, Frydman J, Czymmek K, Chiu W. Multi-scale 3D Cryo-Correlative Microscopy for Vitrified Cells. Structure. 2020 Aug 15;PubMed PMID: 32814034.

b. Wu GH, Smith-Geater C, Galaz-Montoya JG, Gu Y, Gupte SR, Aviner R, Mitchell PG, Hsu J, Miramontes R, Wang KQ, Geller NR, Hou C, Danita C, Joubert LM, Schmid MF, Yeung S, Frydman J, Mobley W, Wu C, Thompson LM, Chiu W. CryoET reveals organelle phenotypes in huntington disease patient iPSC-derived and mouse primary neurons. Nat Commun. 2023 Feb 8;14(1):692. doi: 10.1038/s41467-023-36096-w. PMID: 36754966; PMCID: PMC9908936.

4. **Application of Cryo-ET in investigating mitochondria structure in optic disc drusen**

   I found the mitochondria contain large granules in fibroblast cells treated with calcium calcification medium. Moreover, I established cryo-EELS (electron energy loss spectroscopy) to identify the large mitochondria granules containing Calcium components. I currently focus on using cryo-ET and cryo-EELS to observe and detect mitochondria phenomena and granules component.

Complete list of my published work: https://www.ncbi.nlm.nih.gov/myncbi/gong-her.wu.1/bibliography/public/

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

Ongoing Research Support

Completed Research Support

106-2917-I-564-075, Ministry of Science and Technology, Taiwan
Gong-Her Wu (PI)
08/01/17-11/29/18
Biological Effects of T-complex Protein 1 Ring Complex on Mutated Huntingtin Aggregation in ex vivo and in vitro Huntington's Disease Model
Role: PI

P01NS092525-05, National Institutes of Health
Wah Chiu (PI)
07/01/17-03/31/21
From structure to therapy: the TRiC Chaperonin network in Huntington's disease
Goal: Perform cryo-electron microscopy and tomography for protein aggregates, cells and neurons in the context of Huntington's Disease
Role: Trainee

SPO #150254, Hereditary Disease Foundation Post-Doctoral Fellowship
Gong-Her Wu (PI)
08/01/19-02/28/2023
Deciphering mutated huntingtin aggregates and cellular architecture in Huntington disease neuron by cryogenic electron microscopy
Goal: Deciphering mutated huntingtin aggregates and cellular architecture in both mouse primary and iPSC Huntington disease neurons by cryogenic electron microscopy.
Role: PI

Full Publications

1: Dudek NK, Galaz-Montoya JG, Shi H, Mayer M, Danita C, Celis AI, Viehboeck T, **Wu GH**, Behr B, Bulgheresi S, Huang KC, Chiu W, Relman DA. Previously uncharacterized rectangular bacterial structures in the dolphin mouth. Nat Commun. 2023 Apr 13;14(1):2098. doi: 10.1038/s41467-023-37638-y. PMID: 37055390; PMCID: PMC10102025.

2: **Wu GH**, Smith-Geater C, Galaz-Montoya JG, Gu Y, Gupte SR, Aviner R, Mitchell PG, Hsu J, Miramontes R, Wang KQ, Geller NR, Hou C, Danita C, Joubert LM, Schmid MF, Yeung S, Frydman J, Mobley W, Wu C, Thompson LM, Chiu W. CryoET reveals organelle phenotypes in huntington disease patient iPSC-derived and mouse primary neurons. Nat Commun. 2023 Feb 8;14(1):692. doi: 10.1038/s41467-023-36096-w. PMID: 36754966; PMCID: PMC9908936.

3: Barmaver SN, Muthaiyan Shanmugam M, Chang Y, Bayansan O, Bhan P, **Wu GH**, Wagner OI. Loss of intermediate filament IFB-1 reduces mobility, density, and physiological function of mitochondria in Caenorhabditis elegans sensory neurons. Traffic. 2022 May;23(5):270-286. doi: 10.1111/tra.12838. Epub 2022 Mar 16. PMID: 35261124.

4: **Wu GH**, Mitchell PG, Galaz-Montoya JG, Hecksel CW, Sontag EM, Gangadharan V, Marshman J, Mankus D, Bisher ME, Lytton-Jean AKR, Frydman J, Czymmek K, Chiu W. Multi-scale 3D Cryo-Correlative Microscopy for Vitrified Cells. Structure. 2020 Nov 3;28(11):1231-1237.e3. doi: 10.1016/j.str.2020.07.017. Epub 2020 Aug 18. PMID: 32814034; PMCID: PMC7642057.

5: Li Y, Zhou W, Li Y, Huang W, Zhang Z, Chen G, Wang H, **Wu GH**, Rolston N, Vila R, Chiu W, Cui Y. Unravelling Atomic Structure and Degradation Mechanisms of Organic-Inorganic Halide Perovskites by Cryo-EM. Joule. 2019 Nov 20;3(11):2854-2866. doi: 10.1016/j.joule.2019.08.016. Epub 2019 Aug 28. PMID: 34109301; PMCID: PMC8186345.

6: Li Y, Wang K, Zhou W, Li Y, Vila R, Huang W, Wang H, Chen G, **Wu GH**, Tsao Y, Wang H, Sinclair R, Chiu W, Cui Y. Cryo-EM structures of atomic surfaces and host-guest chemistry in metal-organic frameworks. Matter. 2019 Aug 7;1(2):428-438. doi: 10.1016/j.matt.2019.06.001. Epub 2020 Mar 24. PMID: 34104881; PMCID: PMC8184120.

7: Hu CC, **Wu GH**, Lai SF, Muthaiyan Shanmugam M, Hwu Y, Wagner OI, Yen TJ. Toxic Effects of Size-tunable Gold Nanoparticles on Caenorhabditis elegans Development and Gene Regulation. Sci Rep. 2018 Oct 15;8(1):15245. doi: 10.1038/s41598-018-33585-7. PMID: 30323250; PMCID: PMC6189128.

8: Muthaiyan Shanmugam M, Bhan P, Huang HY, Hsieh J, Hua TE, **Wu GH**, Punjabi H, Lee Aplícano VD, Chen CW, Wagner OI. Cilium Length and Intraflagellar Transport Regulation by Kinases PKG-1 and GCK-2 in Caenorhabditis elegans Sensory Neurons. Mol Cell Biol. 2018 Mar 15;38(7):e00612-17. doi: 10.1128/MCB.00612-17. PMID: 29378827; PMCID: PMC5854826.

9: Hu CC, **Wu GH**, Hua TE, Wagner OI, Yen TJ. Uptake of TiO₂

Nanoparticles into C. elegans Neurons Negatively Affects Axonal Growth and Worm Locomotion Behavior. ACS Appl Mater Interfaces. 2018 Mar 14;10(10):8485-8495. doi: 10.1021/acsami.7b18818. Epub 2018 Mar 5. PMID: 29464946.

10: **Wu GH**, Muthaiyan Shanmugam M, Bhan P, Huang YH, Wagner OI. Identification and Characterization of LIN-2(CASK) as a Regulator of Kinesin-3 UNC-104(KIF1A) Motility and Clustering in Neurons. Traffic. 2016 Aug;17(8):891-907. doi: 10.1111/tra.12413. Epub 2016 Jun 3. PMID: 27172328.

11: Tien NW, **Wu GH**, Hsu CC, Chang CY, Wagner OI. Tau/PTL-1 associates with kinesin-3 KIF1A/UNC-104 and affects the motor's motility characteristics in C. elegans neurons. Neurobiol Dis. 2011 Aug;43(2):495-506. doi: 10.1016/j.nbd.2011.04.023. Epub 2011 May 4. PMID: 21569846.

12: Wagner OI, Esposito A, Köhler B, Chen CW, Shen CP, **Wu GH**, Butkevich E, Mandalapu S, Wenzel D, Wouters FS, Klopfenstein DR. Synaptic scaffolding protein SYD-2 clusters and activates kinesin-3 UNC-104 in C. elegans. Proc Natl Acad Sci U S A. 2009 Nov 17;106(46):19605-10. doi: 10.1073/pnas.0902949106. Epub 2009 Oct 30. PMID: 19880746; PMCID: PMC2780759.

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: Zuchero, J. Bradley (Brad)

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

POSITION TITLE: Assistant Professor of Neurosurgery, Covert-Matera Families Endowed Faculty Scholar

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
Vassar College, Poughkeepsie, NY	B.A.	05/2002	Biology
University of California, San Francisco, CA	Ph.D.	12/2009	Biochemistry & Cell Biology
Stanford University, Stanford, CA	Postdoctoral	12/2016	Neurobiology

A. Personal Statement

My research interest is in understanding the roles of glial cells in health and disease. My primary focus is on the mechanisms driving development, dynamics, and diseases of myelin in the mammalian nervous system. I am an Early Stage Investigator and recently started my lab in the Neurosurgery Department of Stanford University School of Medicine as a tenure track Assistant Professor, and have >90% protected time for research. I trained with leaders in the fields of cytoskeletal cell biology (Dyche Mullins, UCSF/HHMI) and neuron-glia interactions (Ben Barres, Stanford University) and have made several fundamental contributions to both of these fields during my training, resulting in 25 publications that have been cited more than 3900 times. My lab employs a mix of primary culture and in vivo techniques for studying myelination, coupled with high-end imaging techniques and a suite of genetic tools that we created for perturbing and imaging the cytoskeleton.

During my postdoc, I was regularly mentored by Dr. Barres in how to lead and mentor a successful and inclusive research team. As an Assistant Professor, I have taken advantage of numerous opportunities at Stanford for new faculty to learn effective techniques for mentoring postdocs and students. This includes my involvement in SURGE-2 (Starting Up Your Research Group-Advanced), a cohort-based program in Stanford's School of Medicine that aims to help early-career PIs recognize and apply core interactive communication skills, design and implement successful team collaboration, delegate, motivate, manage conflict, and apply inclusive practices effectively. To date, I have mentored 9 PhD students and postdocs in my lab, of whom 1 is a URM and 7 are women.

Together with the support of the Department of Neurosurgery and my numerous collaborations here at Stanford (including Wah Chiu, who will continue to collaborate with my lab and co-sponsor Kathryn's project), Kathryn will have all the expertise and resources required to carry out the research she is proposing.

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

R01 NS119823
Zuchero (PI), Role: PI
09/30/2020-08/31/2025
How Does Actin Disassembly Drive Myelin Wrapping?

R21 NS13199901
Zuchero (PI), Role: PI
07/01/2023-06/30/2025
New cell biology tools to study myelin development, dynamics, and disease

R21 AG084253
Schuele/Zuchero (MPI), Role: MPI
09/01/2023 – 08/31/2025
Developing a cell-on-chip platform to study oligodendrocyte-neuron interactions in plasticity and neurodegeneration

National Multiple Sclerosis Society - Harry Weaver Neuroscience Scholar Award
Zuchero (PI), Role: PI
07/01/2018-06/30/2023
How does the actin cytoskeleton control myelination and remyelination?

The Arnold and Mabel Beckman Foundation - Beckman Young Investigator Award
Zuchero (PI), Role: PI
07/01/2019-06/30/2023
Elucidating new roles of myelin in plasticity, learning and disease

The McKnight Foundation – McKnight Scholar Award
Zuchero (PI), Role: PI
07/01/2018-06/30/2021
Mechanisms of myelin membrane growth and wrapping

Citations:

a. Lam M, Takeo K, Almeida RG, Cooper MH, Wu K, Iyer M, Kantarci H, **Zuchero JB***. CNS myelination requires VAMP2/3-mediated membrane expansion in oligodendrocytes. Nat Commun. 2022;13(1):5583. PMID: 36151203. *Corresponding author

b. Harterink M, Silva ME da, Will L, Turan J, Ibrahim A, Lang AE, Battum EY van, Pasterkamp RJ, Kapitein LC, Kudryashov D, Barres BA, Hoogenraad CC*, **Zuchero JB***. DeActs: genetically encoded tools for perturbing the actin cytoskeleton in single cells. Nat Meth 2017;14(5):479–482. PMID: 28394337. *Co-corresponding author.

c. **Zuchero JB***, Fu M meng, Sloan SA, Ibrahim A, Olson A, Zaremba A, Dugas JC, Wienbar S, Caprariello AV, Kantor C, Leonoudakis D, Leonoudakus D, Lariosa-Willingham K, Kronenberg G, Gertz K, Soderling SH, Miller RH, Barres BA*. CNS myelin wrapping is driven by actin disassembly. Dev Cell [Internet]. 2015 Jul 27;34(2):152–167. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1534580715004001 PMCID: PMC4519368. *Co-corresponding author.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2018-	Member, Stanford Maternal and Child Health Research Institute
2017-	Assistant Professor, Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA
2017-	Faculty Fellow, Stanford ChEM-H
2017-	Member, Stanford Neurosciences Institute
2017-	Member, Stanford Bio-X
2010-2016	Postdoctoral Fellow, Department of Neurobiology, Stanford University School of Medicine, Stanford, CA

Other Experience and Professional Memberships

2023, 2024	Faculty Instructor, Neurobiology Course, Marine Biological Laboratory, Woods Hole
2021-	Course Director, NEPR280: Neuroscience Journal Club & Professional Development Series
2021	Ad hoc reviewer for The Cellular Molecular Biology of Glia (CMBG) Study Section, NIH
2020-	Standing Member, Biomedical Research Committee (B), National MS Society
2020-	Faculty Member, Faculty Opinions (*formerly Faculty of 1000 Prime*)
2020	Reviewer, Peer Reviewed Multiple Sclerosis Research Program (MSRP), CDMRP/DoD
2018-2020	Member, Pilot Glial Grant Review Committee, National MS Society
2018	Session Chair / Discussion Leader, 2018 Myelin Gordon Research Seminar, Ventura, CA
2017-	Ad hoc reviewer for *Science*, *Neuron*, *Nature Methods*, *Nature Neuroscience*, *Nature Communications*, *Developmental Cell*, *PNAS*, *J. Neuroscience*, *eLife*, *J. Cell Biol*, *Cell Reports*, *Plos Genetics*, *Development*, *Biochemistry*, *Glia*, *PlosONE*, *Scientific Reports*, *Science Advances*, *Neural Development*, *JoVE*
2016	Symposium Co-chair, American Society for Neurochemistry, Denver, CO
2016-	Member, American Society for Neurochemistry
2014	Chair, 2014 Myelin Gordon Research Seminar, Ventura, CA
2007-2008	Teaching Assistant, Physiology Course, Marine Biological Laboratory, Woods Hole, MA

Honors

Awards:

2023	MCHRI Faculty Scholar Award, Stanford University
2020	Koret Early Career Award, Stanford University
2019	Beckman Young Investigator Award, Arnold and Mabel Beckman Foundation
2018	McKnight Scholar Award, The McKnight Endowment Fund for Neuroscience
2018	Harry Weaver Neuroscience Scholar Award, National Multiple Sclerosis Society
2014	Discovery Research Award, Myelin Repair Foundation
2012	Pioneer Award, Myelin Repair Foundation
2002	Virginia Swinburne Brownell Prize for excellent work in biology, Vassar College
2002	Phi Beta Kappa, Vassar College
2000	Harriet Gurnee van Allen Prize for Excellence in Biology, Vassar College

Fellowships:

2015-2019	Career Transition Award, National Multiple Sclerosis Society
2011-2014	Postdoctoral Fellowship, Life Sciences Research Foundation
2011	Postdoctoral Fellowship, National Multiple Sclerosis Society (declined)
2010-2011	Fellowship, Developmental & Neonatal Biology Training Program, Stanford University
2009	Robert Day Allen Fellowship, Marine Biological Laboratory, Woods Hole, MA
2005-2009	Predoctoral Fellowship, American Heart Association
2004-2005	Genentech Fellowship, University of California, San Francisco

C. Contributions to Science

1. **Actin cytoskeleton regulation and genetic tools to study actin.** My graduate work with Prof. Dyche Mullins at UCSF focused on understanding how cells build complex cytoskeletal structures to change their morphology and move. At the start of my PhD only two types of actin nucleation factors—proteins that nucleate the formation of actin filaments from cytoplasmic actin monomers—had been discovered: formins and the Arp2/3 complex. I discovered that the p53-cofactor JMY is a novel actin nucleation factor. Further, I discovered that JMY contributes to cell motility and is regulated by shuttling between the nucleus and cytoplasm. These studies established JMY as a novel, vertebrate-specific regulator of the actin cytoskeleton, and JMY subsequently became a major focus of the Mullins lab and other labs.

   At the time of starting my own lab, existing tools for directly manipulating actin dynamics were limited to cell-permeable drugs, which cannot be used to understand the role of actin filaments in specific cell types (e.g. oligodendrocytes) within multicellular organisms. To overcome this limitation, I created “DeActs,” a set of genetically encoded tools that potently and specifically induce actin disassembly in cells. In collaboration

with Dr. Casper Hoogenraad, we showed that DeActs can be used to selectively ablate actin filaments in vivo, e.g. in a specific pair of sensory neurons in *C. elegans*, or in neuronal precursors in the developing mouse CNS. We made these constructs available through Addgene which to date has distributed more than 250 samples to researchers worldwide.

a. **Zuchero, J.B.**, Coutts, A., Quinlan, M., La Thangue, N., and Mullins, R.D. (2009). p53-cofactor JMY is a Multifunctional Actin Nucleation Factor. *Nature Cell Biology* 11, 451-459. PMCID: PMC2763628.
b. **Zuchero, J.B.**, Belin, B., and Mullins, R.D. (2012). Actin Binding to WH2 Domains Regulates Nuclear Import of the Multifunctional Actin Regulator JMY. *Molecular Biology of the Cell* 23, 853–863. PMCID: PMC3290644.
c. **Zuchero, J.B.*** (2007) In vitro Actin Assembly Assays and Purification from Acanthamoeba. *Methods in Molecular Biology* 370, 213-226. PMCID: N/A.
d. Harterink, M., Silva, M., Hoogenraad, C.C.*, and **Zuchero, J.B.*** (2017). DeActs: genetically encoded tools for perturbing the actin cytoskeleton in single cells. *Nature Methods* 14, 479–482. PMCID: PMC5419720.

***Corresponding author(s)**

2. **Cellular mechanisms of myelination**. My postdoctoral work with Prof. Ben Barres at Stanford brought my interests and expertise in the cytoskeleton to the study of how oligodendrocytes myelinate axons in the central nervous system. Myelin is essential in vertebrates for rapid nerve conduction, but the cellular mechanisms that drive myelination have remained unknown. At the start of my postdoc, the leading hypothesis in the field was that actin assembly powers myelin wrapping, similar to the lamellipodium of a motile cell. Surprisingly, I discovered that myelin wrapping represents a novel form of cell motility driven by the complete disassembly of the oligodendrocyte actin cytoskeleton. Based on my discoveries, I proposed a new cellular model of myelin wrapping that has profound implications for how myelin is generated during development. In my own lab, we have also expanded our cell biology focus to ask how new membrane is added to form and tune myelin, and discovered that regulated exocytosis by Vamp2 and Vamp3 is essential for CNS myelination. Moreover, in unpublished work that is currently under invited re-review, we uncovered oligodendrocyte calcium signaling as a regulator of developmental myelination (Iyer et al. 2023, bioRxiv). These discoveries open the doors to understanding how myelin sheaths tune themselves to neuronal properties during development and as part of learning—e.g. by calcium-regulated exocytosis.
    a. **Zuchero JB***, Fu MM, Sloan SA, Ibrahim A, Soderling SH, Miller RH, Barres BA*. (2015) CNS myelin wrapping is driven by actin disassembly. *Developmental Cell* 34, 152-167. PMCID: PMC4519368.
    b. **Zuchero JB***, Barres, BA*. (2015) Glia in mammalian development and disease. *Development* 142, 3805-3809. PMCID: PMC4712885.
    c. Lam M, Takeo K, Almeida RG, Cooper MH, Wu K, Iyer M, Kantarci H, & **Zuchero JB***. (2022) CNS myelination requires VAMP2/3-mediated membrane expansion in oligodendrocytes. *Nature Communications* 13, 5583. PMID: 36151203.
    d. Iyer M, Kantarci H, Ambiel N, Novak SW, Andrade LR, Lam M, Munch AE, Yu X, Khakh BS, Manor U, & **Zuchero JB***. Oligodendrocyte calcium signaling sculpts myelin sheath morphology. *bioRxiv* 2023.04.11.536299 **[Preprint]**. April 12, 2023 [cited 2023 April 15]. Available from: https://doi.org/10.1101/2023.04.11.536299

    ***Corresponding author(s)**

3. **A novel role of glia in promoting neuronal excitability during development**. How do neurons first become electrically excitable during development? By creating a method to rapidly purify embryonic rodent sensory neurons away from glia, we serendipitously discovered that purified neurons were hypoexcitable and failed to produce normal trains of action potentials. Adding back Schwann cells (the myelinating glia that normally coat all sensory neurons' axons in vivo) or even media "conditioned" by Schwann cells rapidly restored neuronal excitability. Biochemical fractionation revealed the active secreted molecule to be the prostaglandin PGE_2. We found that Schwann cell-derived PGE_2 is necessary and sufficient for embryonic sensory neurons to express normal levels of voltage-gated sodium channels to achieve mature excitability.

We identified Ptges3 as the required PGE_2-synthesizing enzyme in Schwann cells. Incredibly, conditionally knocking out *Ptges3* selectively in Schwann cells drastically reduces the expression of Na_Vs in DRG neurons in vivo and cause corresponding sensory deficits. Together, our data show that glia actively promote the maturation of sensory neurons into excitable cells through secretion of PGE_2. Our findings also raise the possibility that this novel function of glia is conserved across the nervous system.

a. **Zuchero JB**. (2014) Purification of Dorsal Root Ganglion Neurons from Rat by Immunopanning. *Cold Spring Harb Protoc* 2014(8), 826-838. PMCID: PMC4438770.
b. **Zuchero JB**. Purification and culture of dorsal root ganglion neurons. *Cold Spring Harb Protoc*. 2014 Aug 1;2014(8):813–4. PMCID: PMC4438772
c. Kantarci H, Elvira PD, Thottumkara AP, Iyer M, Donovan LJ, Ambiel A, O'Connell EM, Granados A, Zeng H, Saw NL, Lutz AB, Sloan SA, Gray EE, Tran KV, Vichare A, Yeh AK, Münch AE, Huber M, Agrawal A, Morri M, Shamloo M, Tawfik VL, Du Bois J*, **Zuchero JB***. Schwann cells promote sensory neuron excitability during development. *bioRxiv* 2022.10.31.514415 **[Preprint]**. October 31, 2022 [cited 2022 Nov 1. Available from: https://doi.org/10.1101/2022.10.31.514415

***Corresponding author(s)**

4. **Collaborative work in the myelin field.** I have contributed my expertise in myelin biology to several collaborative projects aimed at answering fundamental questions in the field, including: (1) Can we reprogram fibroblast cells into oligodendrocytes for disease modeling or therapies? (2) How do oligodendrocytes respond to neuronal activity? (3) What is the cause of multiple sclerosis? (4) What is the role of myelin dysfunction in aging-related diseases including neurodegeneration?

a. Yang, N., **Zuchero, J.B.**, Ahlenius, H., Marro, S., Ng, Y.H., Vierbuchen, T., Hawkins, J.S., Barres, B.A., and Wernig, M. (2013). Generation of oligodendroglial cells by direct lineage conversion. *Nature Biotechnology* 31, 434-439. PMCID: PMC3677690.

b. Gibson, E.M., Purger, D., Mount, C.W., Goldstein, A.K., Lin, G.L., Wood, L.S., Inema, I., Miller, S.E., Bieri, G., **Zuchero, J.B.**, Barres, B.A., Woo, P.J., Vogel, H. & Monje, M. (2014). Neuronal Activity Promotes Oligodendrogenesis and Adaptive Myelination in the Mammalian Brain. *Science* 344, 1252304. PMCID: PMC4096908.

c. Lanz TV, Brewer RC, Ho PP, Moon J-S, Jude KM, Fernandez D, Fernandes RA, Gomez AM, Nadj G-S, Bartley CM, Schubert RD, Hawes IA, Vazquez SE, Iyer M, **Zuchero JB**, Teegen B, Dunn JE, Lock CB, Kipp LB, Cotham VC, Ueberheide BM, Aftab BT, Anderson MS, DeRisi JL, Wilson MR, Bashford-Rogers RJM, Platten M, Garcia KC, Steinman L, Robinson WH. Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM. *Nature* 603, 321–327. PMCID: PMC9382663.

d. Iram T, Kern F, Kaur A, Myneni S, Morningstar AR, Shin H, Garcia MA, Yerra L, Palovics R, Yang AC, Hahn O, Lu N, Shuken SR, Haney MS, Lehallier B, Iyer M, Luo J, Zetterberg H, Keller A, **Zuchero JB**, Wyss-Coray T. (2022) Young CSF restores oligodendrogenesis and memory in aged mice via Fgf17. *Nature* 605, 509–515. PMCID: PMC9377328.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1Zqp957kev/bibliography/public/