BIOGRAPHICAL SKETCH

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NAME: Zhao, Minglei

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

POSITION TITLE: Assistant Professor of Biochemistry and Molecular Biology

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
Fudan University, Shanghai	BS	06/2005	Biological Sciences
University of California Los Angeles, Los Angeles, California	PHD	06/2011	Molecular Biology
Stanford University, Stanford, California	Postdoctoral Fellow	12/2016	Structural Biology / Biophysics

A. Personal Statement

As a structural biology lab, we are interested in understanding the mechanism of molecular machines and their roles in various diseases. Currently we focus on two systems, p97 / ubiquitination system, and Vault. p97 is a central hub in cellular protein homeostasis. It is involved in several neurodegenerative diseases, and is also a cancer drug target. We want to gain insights into the molecular architectures of p97 in complex with various adaptor proteins and poly-ubiquitinated substrates. We are investigating the preference of p97 towards specific ubiquitin chains. Our findings will nurture new approaches to tackle the diseases. Vault is the largest ribonuclear protein in many eukaryotes including human. It can be regarded as a membraneless organelle. Despite the fact that Vault has been discovered for thirty years, the function of vault remains elusive. Overexpression of major vault protein (MVP) correlates with drug resistance in cancer cells. However, the mechanism is completely unknown at the molecular level. We are investigating the structures of Vault components, and using proteomic and imaging techniques to address the molecular function of Vault. Two major techniques are used in the lab: X-ray crystallography and cryo-electron microscopy. Throughout my graduate and postdoc research, I have got extensive training in both techniques. Combining the two techniques will enable us to elucidate the molecular mechanism of p97 and vault.

B. Positions and Honors

Positions and Employment

2012 - 2014 Postdoctoral Associate, Stanford University, Stanford, CA
2014 - 2016 Research Associate, Howard Hughes Medical Institute, Stanford, CA
2017 - Assistant Professor of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL

Other Experience and Professional Memberships

2010 - Member, American Crystallographic Association
2014 - Member, Biophysical Society

Honors

2009 Philip J. Whitcome Fellowship, University of California Los Angeles
2010 Dissertation Year Fellowship, University of California Los Angeles
2018 Chicago Biomedical Consortium Catalyst Award

C. Contribution to Science

1. Structural studies of protein complexes involved in vesicle and membrane fusion

I studied structures of protein complexes involved in vesicle and membrane fusion for my postdoctoral research. Vesicle and membrane fusion are essential for many physiological processes in eukaryotic cells, including protein trafficking, hormone secretion, and neurotransmitter release. The publications represent the efforts to elucidate the mechanisms of these intricate machineries using either X-ray crystallography or single-particle cryoEM as the major technique. The structures of full-length NSF (N-ethylmaleimide sensitive factor) and its complex with adaptor and substrate proteins (20S supercomplex) are considered as a milestone in a long history of structural studies in this field. It is actually the first time that a protein-disassembling machine has been visualized with its substrate at near-atomic to sub-nanometer resolutions. It also demonstrated a striking molecular asymmetry of the hexameric AAA+ ATPases which had been modeled as six-fold symmetric. Besides the NSF and 20S supercomplex, I also made key contributions to the structural studies of synaptotagmin-SNARE complex and autophagic SNARE complex, which are important protein machinery involved in neurotransmission and autophagy.

a. **Zhao M**, Wu S, Zhou Q, Vivona S, Cipriano DJ, Cheng Y, Brunger AT. Mechanistic insights into the recycling machine of the SNARE complex. Nature. 2015 Feb 5;518(7537):61-7. PubMed PMID: 25581794; PubMed Central PMCID: PMC4320033.
b. White KI, **Zhao M**, Choi UB, Pfuetzner RA, Brunger AT. Structural principles of SNARE complex recognition by the AAA+ protein NSF. Elife. 2018 Sep 10;7. doi: 10.7554/eLife.38888. PubMed PMID: 30198481; PubMed Central PMCID: PMC6160233.
c. Zhou Q, Zhou P, Wang AL, Wu D, **Zhao M**, Südhof TC, Brunger AT. The primed SNARE-complexin-synaptotagmin complex for neuronal exocytosis. Nature. 2017 Aug 24;548(7668):420-425. PubMed PMID: 28813412; PubMed Central PMCID: PMC5757840.
d. Diao J, Liu R, Rong Y, **Zhao M**, Zhang J, Lai Y, Zhou Q, Wilz LM, Li J, Vivona S, Pfuetzner RA, Brunger AT, Zhong Q. ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes. Nature. 2015 Apr 23;520(7548):563-6. PubMed PMID: 25686604; PubMed Central PMCID: PMC4442024.

2. Elucidating the mechanism of synaptic vesicle fusion using single molecule approach

I contributed to the mechanistic study of synaptic vesicle fusion using single-molecule fluorescence microscopy techniques.

a. Choi UB, **Zhao M**, White KI, Pfuetzner RA, Esquivies L, Zhou Q, Brunger AT. NSF-mediated disassembly of on- and off-pathway SNARE complexes and inhibition by complexin. Elife. 2018 Jul 9;7. doi: 10.7554/eLife.36497. PubMed PMID: 29985126; PubMed Central PMCID: PMC6130971.
b. Lai Y, Choi UB, Leitz J, Rhee HJ, Lee C, Altas B, **Zhao M**, Pfuetzner RA, Wang AL, Brose N, Rhee J, Brunger AT. Molecular Mechanisms of Synaptic Vesicle Priming by Munc13 and Munc18. Neuron. 2017 Aug 2;95(3):591-607.e10. PubMed PMID: 28772123; PubMed Central PMCID: PMC5747255.
c. Lai Y, Choi UB, Zhang Y, **Zhao M**, Pfuetzner RA, Wang AL, Diao J, Brunger AT. N-terminal domain of complexin independently activates calcium-triggered fusion. Proc Natl Acad Sci U S A. 2016 Aug 9;113(32):E4698-707. PubMed PMID: 27444020; PubMed Central PMCID: PMC4987820.
d. Choi UB, **Zhao M**, Zhang Y, Lai Y, Brunger AT. Complexin induces a conformational change at the membrane-proximal C-terminal end of the SNARE complex. Elife. 2016 Jun 2;5PubMed PMID: 27253060; PubMed Central PMCID: PMC4927292.

3. Structural studies of amyloid-forming proteins and peptides

I studied structures of amyloid proteins and peptides using X-ray crystallography as a major technique for my graduate research. Many neurodegenerative diseases are associated with deposition of insoluble plaques of amyloid proteins including the well-known Alzheimer's disease and Parkinson's disease. Elucidating the structures of these plaques is of central importance to understand the etiology of the diseases and to develop drugs for treatment. Insoluble fibrils and soluble oligomers had both been proposed to be the

toxic species, but the structural information at atomic level was elusive. While many publications are more than five years, I am still collaborating with Cong Liu group at IRCBC on the determination of the crystal structures of amyloid peptides using either X-ray crystallography or microED, which is often technically challenging. Listed are two recent publications.

a. Gui X, Luo F, Li Y, Zhou H, Qin Z, Liu Z, Gu J, Xie M, Zhao K, Dai B, Shin WS, He J, He L, Jiang L, **Zhao M**, Sun B, Li X, Liu C, Li D. Structural basis for reversible amyloids of hnRNPA1 elucidates their role in stress granule assembly. Nat Commun. 2019 May 1;10(1):2006. doi: 10.1038/s41467-019-09902-7. PubMed PMID: 31043593; PubMed Central PMCID: PMC6494871.
b. Luo F, Gui X, Zhou H, Gu J, Li Y, Liu X, **Zhao M**, Li D, Li X, Liu C. Atomic structures of FUS LC domain segments reveal bases for reversible amyloid fibril formation. Nat Struct Mol Biol. 2018 Apr;25(4):341-346. PubMed PMID: 29610493.

4. Method development for X-ray free electron laser

I contributed to the methods development for serial crystallography using X-ray free electron laser (XFEL).

a. Lyubimov AY, Uervirojnangkoorn M, Zeldin OB, Zhou Q, **Zhao M**, Brewster AS, Michels-Clark T, Holton JM, Sauter NK, Weis WI, Brunger AT. Advances in X-ray free electron laser (XFEL) diffraction data processing applied to the crystal structure of the synaptotagmin-1 / SNARE complex. Elife. 2016 Oct 12;5PubMed PMID: 27731796; PubMed Central PMCID: PMC5094853.
b. Zeldin OB, Brewster AS, Hattne J, Uervirojnangkoorn M, Lyubimov AY, Zhou Q, **Zhao M**, Weis WI, Sauter NK, Brunger AT. Data Exploration Toolkit for serial diffraction experiments. Acta Crystallogr D Biol Crystallogr. 2015 Feb;71(Pt 2):352-6. PubMed PMID: 25664746; PubMed Central PMCID: PMC4321488.

Complete List of Published Work (29 peer reviewed papers in total):
https://www.ncbi.nlm.nih.gov/myncbi/minglei.zhao.1/bibliography/public/

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

Pending Research Support

1DP2OD031009 ZHAO MINGLEI (PI) 08/15/2021-06/30/2026
Elucidating the Function of Vault at Molecular, Cellular, and Organismal Levels
The goal of this study is to systematically examine Vault's function in innate immune response and drug resistance using imaging and omics approaches.
Role: PI

1R35GM143052 ZHAO MINGLEI (PI) 07/01/2021-06/30/2026
Structural and Functional Studies of Molecular Machines Involved in Chemical Modifications of Macromolecules
The goal of this study is to study the p97 related ubiquitination system and Vault related ADP-ribosylation system using structural and chemical biology approaches.
Role: PI

BIOGRAPHICAL SKETCH

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NAME: Aaron P. Turkewitz

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

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
Harvard University, Cambridge, MA	B.A.	1981	Biochem.
Harvard University, Cambridge, MA	Ph.D.	1988	Biochem. & Mol. Biol.
UCSF, San Francisco, CA	Postdoc	1988-1993	Cell Biology

A. Personal Statement

My laboratory exploits the genetic accessibility of the ciliate *Tetrahymena thermophila*, combined with its exceptional accessibility for microscopy and a variety of cell biological approaches, to ask both mechanistic and evolutionary questions about pathways in eukaryotic membrane traffic, with a particular focus on the biogenesis of secretory granules and lysosome-related organelles. In the course of our work we have also developed tools to advance the community. Most recently, we developed an automated approach to exploiting the extensive publicly-accessible transcriptome data for *T. thermophila*, which also helps overcome problems with incomplete annotation of the *T. thermophila* genome. The program, called the Coregulation Data Harvester, is hosted on the Tetrahymena Genome website. Judging by personal communication, it has proven valuable for numerous ciliate researchers and also for scientists study the related Apicomplexan parasites, *Toxoplasma gondii* and *Plasmodium falciparum*.

I am a member of an international committee, and former elected Chairman, to discuss and oversee issues related to research and education in my field. Called the Tetrahymena Research Advisory Board, the group was initially focused on Tetrahymena but has since expanded to include all ciliates. We help organize meetings and workshops, and meet at least monthly via Zoom to discuss initiatives for expanding community resources.

I am PI and director of an NSF-funded Research Experiences for Undergraduates (REU) Program in Molecular Genetics and Cell Biology at the University of Chicago. I initiated this program in 2009 to increase research opportunities for undergraduates at institutions without advanced (i.e., PhD-level) molecular cell biology laboratories, particularly undergraduates from traditionally under-represented minority groups.

B. Positions and Honors

Positions

9/81-6/84 Predoctoral Fellow, Biochemistry Department, Harvard Univ. (Dr. Stephen Harrison)
3/89-8/92 Postdoctoral Fellow, Biochem. & Biophysics, UCSF (Dr. Regis Kelly)
9/92-2/93 Visiting Research Fellow, Neurobiology, CNRS, Rome, Italy
3/93-12/00 Assistant Professor, Molecular Genetics & Cell Biology, University of Chicago
1/01-6/14 Associate Professor, Molecular Genetics & Cell Biology, University of Chicago
7/14-present Professor, Molecular Genetics & Cell Biology, University of Chicago

2002	Organizer, Midwest Protozoology Conference
2004	Co-organizer, Minisymposium on Regulated Secretion, American Society for Cell Biology Annual Meeting
2007	Program Committee Member, Annual Meeting of the American Society for Cell Biology
2012	Co-organizer (with H. Goodson, M. Lynch) NSF-sponsored meeting on "Defining the Field of Evolutionary Cell Biology", Airlie Center, Virginia.
2013	Primary organizer, FASEB Conference on Ciliate Molecular Biology, Colorado Springs
2013	Organizer, symposium on Evolutionary Protistology for International Congress of Protistology
2004-present	Board of reviewers – Journal of Eukaryotic Microbiology
2006-2016	Editorial board – Eukaryotic Cell
2007-2010	Member, NIH ICP1 Study Section
2009-present	PI, Research Experiences for Undergraduates Program in Molecular Genetics & Cell Biology
2014-present	Elected member and current chair, Tetrahymena Research Advisory Board
Various	Manuscript reviewer for Aquatic Microbial Ecology, Biochimica et Biophysica Acta, BMC Biotechnology, BMC Microbiology, Comp. Biochem. Physiol. C Toxicol. Pharmacol., Current Biology, Eukaryotic Cell, European Journal of Cell Biology, Gene, Genetics, Journal of Biological Chemistry, Journal of Cell Biology, Journal of Cell Science, Journal of Eukaryotic Microbiology, Journal of Clinical Investigation, Journal of Molecular Evolution, Molecular and Cellular Biology, Molecular Biology of the Cell, Molecular Cell, Molecular Microbiology, Nature Biotechnology, Nature Nanotubes, PeerJ, PLoS Biology, PLoS Genetics, PLoS Microbiology, PLoS One, Protist, Traffic

Honors, Awards, Other Experience

NSF Predoctoral Fellowship (1981-1984)
Helen Hay Whitney Postdoctoral Fellowship (1989-1992)
American Cancer Society Postdoctoral Fellowship (1992)
NSF-NATO research fellow (1992-1993)
University of Chicago Graduate Teaching/Mentorship Award (2001)
Fulbright Specialist Program (2016-2021)

C. Contribution to Science

1. My early contributions were directed at biochemical analysis of the human transferrin receptor, which is critical for iron uptake at the cellular level. I developed an efficient procedure for purifying large quantities of the receptor, and then measured a set of basic physical parameters. The reagents I developed in the course of this work allowed me to estimate the occupancy by transferrin receptors within clathrin coated vesicles during their endocytosis, and to demonstrate that the binding of transferrin to its receptor was likely to modulate receptor clustering.

Turkewitz, A.P., Amatruda, J.F., Borhani, D., Harrison, S.C. and A.L. Schwartz (1988) A high-yield purification of the human transferrin receptor and properties of its major extracellular fragment. J. Biol. Chem. 263: 8318-8325.

Turkewitz, A.P., Schwartz, A.L. and S.C. Harrison (1988) A pH-dependent reversible conformational transition in the human transferrin receptor leads to self-association. J. Biol. Chem. 263: 16309-16315.

Turkewitz, A.P. and S.C. Harrison (1989) Concentration of transferrin receptor in human placental coated vesicles. J. Cell Biol. 108: 2127-2135.

2. I developed the ciliate *Tetrahymena thermophila* as a genetically tractable model system for studying the biogenesis of secretory granules. Secretory granules play critical roles in many animal tissues, but basic questions about the mechanisms underlying their formation remain unanswered. With a team of collaborators, I identified a broad set of genes underlying secretory granule formation in *Tetrahymena*, and continue to study the underlying mechanisms. Our approaches have included developing mutant screens, and using whole genome sequencing to identify the molecular lesions, which overcomes a long-standing hurdle in ciliate genetics. Our recent work has uncovered unexpected similarities with the mechanisms involved in formation of lysosome-related organelles.

Chilcoat, N.D., N.C. Elde and A.P. Turkewitz (2001) An antisense approach to phenotype-based gene cloning in Tetrahymena. Proc. Natl. Acad. Sci. USA 98: 8709-8713.

Briguglio, J.S., Kumar, S., and A.P. Turkewitz (2013) Lysosomal sorting receptors are essential for secretory granule biogenesis in Tetrahymena. *J. Cell Biol*. 203: 537-50. PMCID: PMC3824020

Kaur, H., Sparvoli, D., Osakada, H., Iwamoto, M., Haraguchi, T., and A.P. Turkewitz (2017) An endosomal syntaxin and the AP-3 complex are required for the formation and maturation of candidate lysosome-related organelles (mucocysts) in Tetrahymena thermophila. Mol. Biol. Cell **28**: 1551-64.

Sparvoli, D., Richardson, E., Osakada, H., Lan, X., Iwamoto, M., Bowman, G.R., Kontur, C., Bourland, W.A., Lynn, D.H., Pritchard, J.K., Haraguchi, T., Dacks, J.B., and Turkewitz, A.P. (2018) Remodeling the specificity of an endosomal CORVET tether underlies formation of regulated secretory vesicles in the ciliate *Tetrahymena thermophile.* Curr. Biol. **28**: 697-710.

3. By studying mechanisms underlying endocytosis in *Tetrahymena*, my laboratory discovered that a key gene had evolved via an unexpected trajectory, in which it independently acquired the same function as an animal cell gene in the analogous pathway. This finding led us to reconsider classical paradigms for the evolution of cell biological pathways, examining this issue for other gene families. Our unique expertise in ciliate membrane trafficking has also allowed us to contribute to multi-organism analyses of such evolutionary questions.

Elde, N.C., Morgan, G., Winey, M., Sperling, L., and A.P. Turkewitz (2006) Elucidation of clathrin-mediated endocytosis in Tetrahymena reveals an evolutionarily convergent recruitment of dynamin. PLoS Genetics 1(5): e52.

Bright, L., Kambesis, N., Nelson, S.B. and A.P. Turkewitz (2010) Comprehensive analysis reveals dynamic and evolutionary plasticity of Rab GTPases and membrane traffic in *Tetrahymena thermophila*. PLOS Genetics 6(10): e1001155. PMCID: PMC2954822

Lynch, M., Field, M.C., Goodson, H., Malik, H.S., Pereira-Leal, J.B., Roos, D. S., Turkewitz, A.P., and S. Sazer (2014) Evolutionary Cell Biology: Two Origins, One Objective. Proc. Natl. Acad. Sci. 111: 16990-4.

Klinger, C.M., Ramirez-Macias, I., Herman, E.K., Turkewitz, A.P., Field, M.C., and J.B. Dacks (2016) Resolving the homology-function relationship through comparative genomics of membrane-trafficking machinery and parasite cell biology. Mol. & Biochem. Parasitol. 209: 88-103.

Sparvoli, D., Zoltner, M., Cheng, C.-Y., Field, M.C., and A.P. Turkewitz (2020) Diversification of CORVET tethers facilitates transport complexity in Tetrahymena thermophila. J. Cell Sci. 133: PMCID: PMC7033735.

4. The extensive and well-curated transcriptomic data for *T. thermophila* represent a valuable asset for biologists. However, accessing and interpreting these data is limited by the non-reliable current annotation of the genome, which often makes it difficult to securely identify a gene (i.e., identify its true homologs in other organisms) without extensive additional work. To overcome these issues, we created an automated approach to mining transcriptomic data and identifying orthologous genes. The program we created appears to be actively used, including by scientists who are studying the related Apicomplexan parasites, *Toxoplasma gondii* and *Plasmodium falciparum*.

Tsypin, L.M. and A.P. Turkewitz (2017) The Co-regulation Data Harvester: automating gene annotation starting from a transcriptome database. SoftwareX 6: 165-171.

5. We have regularly provided help to colleagues who are interested in using Tetrahymena but lack the expertise. This includes sending cell lines, often with GFP- or mCherry-tagged proteins, to undergraduates who request them for student projects. In 2017/8, we have thus far sent cells and detailed protocols to undergraduates at three institutions. We also regularly provide training and technical help to other research groups, facilitating their work with Tetrahymena. We are currently engaged in three such efforts, with

laboratories whose own expertises are in evolutionary biology (primarily using animal cells), apicomplexans (using Toxoplasma and Plasmodium), and Drosophila.

Recent collaborations with two other groups focused on gene regulation. One study focused on coordination of methallothionein genes, while the 2nd examined the role of N^6-methyldeoxyadenosine in nucleosome positioning. Manuscripts arising from that work are listed below:

de Francisco P, Martín-González A, Turkewitz AP, Gutiérrez JC. 2017. Extreme metal adapted, knockout and knockdown strains reveal coordinated gene expression among different *Tetrahymena thermophila* metallothionein isoforms. PLoS One 12: e0189076

de Francisco P, Martín-González A, Turkewitz AP, Gutiérrez JC. 2018. Genome plasticity in response to stress in *Tetrahymena thermophila*: selective and reversible chromosome amplification and paralogous expansion of metallothionein genes. Environ Microbiol. 20: 2410-2421. PMCID: PMC6117198

Luo GZ, Hao Z, Luo L, Shen M, Sparvoli D, Zheng Y, Zhang Z, Weng X, Chen K, Cui Q, Turkewitz A, He C. 2018. N^6-methyldeoxyadenosine directs nucleosome positioning in Tetrahymena DNA. Genome Biol. 19: 200.

Complete List of Published Work:
https://www.ncbi.nlm.nih.gov/pubmed/?term=turkewitz+a

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

Ongoing Research Support

NIH/NIGMS 2RO1GM105783-05 Turkewitz (PI) 04/01/2019-03/31/2023
"Mechanisms of tether function in endolysosomal trafficking"
Project goals: to utilize a combination of biochemical analysis, targeted mutagenesis, and light microscopy to analyze tether subunits expressed at endogenous levels. Correlative light and electron microscopy will be used to visualize the ultrastructure of membrane compartments decorated by tagged proteins, allowing for detailed in vivo analysis of tether function.

NSF MCB-1937326 Turkewitz (PI) 02/01/2020-01/31/2023
"Architecture of endolysosomal pathways in Tetrahymena"
Project goals: To analyze the pathways involved in delivery of proteins to two different compartments in the ciliate Tetrahymena thermophila

Completed Research Support (past 3 years)

NSF MCB-1613922 Turkewitz (PI) 08/01/2016-07/31/2018
"Forward genetic analysis of lysosome-related organelle formation in *Tetrahymena thermophila*"
Project goals: Using whole genome sequencing to identify the genetic lesions in a collection of Tetrahymena mutants.

NIH 1R01GM105783-01A1 Turkewitz (PI) 05/01/2014-4/01/2018
"Sortilin-dependent traffic to dense core secretory granules"
Project goals: To understand the role of Vps10-family and other LRO-associated proteins in mucocyst formation

BIOGRAPHICAL SKETCH

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NAME: Yu-Yang Jiang

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

POSITION TITLE: Postdoctoral 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
Zhejiang University, Hangzhou, China	BS	06/2009	Biological Science
University of Georgia, Athens, GA US	PHD	05/2017	Cellular Biology
University of Georgia, Athens, GA US	postdoc	06/2020	Cellular Biology

A. Personal Statement

I have 11 years of training in cellular biology research. Most of my work used *Tetrahymena thermophila* as laboratory model. I have broad knowledge and practice in cell biology and molecular biology. I successfully transferred total internal reflection fluorescence microscopy technique to *Tetrahymena* allowing live imaging of fluorescence tagged proteins at or near the cell surface [1]. I have extensive experience in genetic engineering in *Tetrahymena* including tagging genes and localizing gene product [2,3]. I developed the first genetic suppressor screen in *Tetrahymena* based on whole genome sequencing [4]. Developing such tools require broad collaboration and significant investment in research time. I believe that my performance and experience in cell biology and genetics of *Tetrahymena* make me valuable personnel to this application.

1. Jiang YY, Lechtreck K, Gaertig J. Total internal reflection fluorescence microscopy of intraflagellar transport in Tetrahymena thermophila. Methods Cell Biol. 2015;127:445-56. doi: 10.1016/bs.mcb.2015.01.001. PMID: 25837403.
2. Jiang YY, Maier W, Baumeister R, Minevich G, Joachimiak E, Ruan Z, et al. The Hippo Pathway Maintains the Equatorial Division Plane in the Ciliate *Tetrahymena*. Genetics. 2017. doi: 10.1534/genetics.117.200766. PMID: 28413159.
3. Jiang YY, Maier W, Baumeister R, Joachimiak E, Ruan Z, Kannan N, et al. Two Antagonistic Hippo Signaling Circuits Set the Division Plane at the Medial Position in the Ciliate *Tetrahymena*. Genetics. 2018. Epub 2018/12/30. doi: 10.1534/genetics.118.301889. PMID: 30593491.
4. Jiang YY, Maier W, Baumeister R, Minevich G, Joachimiak E, Wloga D, et al. LF4/MOK and a CDK-related kinase regulate the number and length of cilia in Tetrahymena. PLoS genetics. 2019;15(7):e1008099. Epub 2019/07/25. doi: 10.1371/journal.pgen.1008099. PMID: 31339880.

B. Positions and Honors

2009-2017 Graduate Research Assistant, University of Georgia, Athens, GA
2017-2020 Postdoctoral Research Associate, University of Georgia, Athens, GA
2020-present Postdoctoral Scholar, University of Chicago, Chicago, IL

C. Contributions to Science

1. Transferring Total Internal Reflection Fluorescence Microscopy (TIRFM) technique to *Tetrahymena*. TIRFM allows imaging fluorescence tagged subject at or near the specimen surface. This technique has been proven powerful in cell biology research as it provides single molecule resolution and allows accurate characterization of protein movement over a long distance. Until recently, TIRFM has been used only in a select few eukaryotic model organisms. I adopted existing TIRFM technique from *Chlamydomonas reinhardtii* and developed method suitable to live image fluorescence tagged proteins in *Tetrahymena*. Initial report of this

work characterized movement of intraflagellar transport (IFT) protein in cilia of *Tetrahymena* [1]. Further application of TIRFM in *Tetrahymena* has produced supporting evidence to reveal IFT behavior common to multiple species [2]. In addition, this method has been employed in several publications that are unrelated to my research project [3, 4].

1. Jiang YY, Lechtreck K, Gaertig J. Total internal reflection fluorescence microscopy of intraflagellar transport in *Tetrahymena thermophila*. Methods Cell Biol. 2015;127:445-56. doi: 10.1016/bs.mcb.2015.01.001. PubMed PMID: 25837403.
2. Wingfield JL, Mengoni I, Bomberger H, Jiang Y-Y, Walsh JD, Brown JM, et al. IFT trains in different stages of assembly queue at the ciliary base for consecutive release into the cilium. eLife. 2017;6:e26609. doi: 10.7554/eLife.26609.
3. Vasudevan KK, Jiang YY, Lechtreck KF, Kushida Y, Alford LM, Sale WS, et al. Kinesin-13 regulates the quantity and quality of tubulin inside cilia. Mol Biol Cell. 2015;26(3):478-94. doi: 10.1091/mbc.E14-09-1354. PMID: 25501369.
4. Louka P, Vasudevan KK, Guha M, Joachimiak E, Wloga D, Tomasi RF, et al. Proteins that control the geometry of microtubules at the ends of cilia. The Journal of cell biology. 2018. doi: 10.1083/jcb.201804141. PMID: 30217954.

2. Developing first genetic suppressor screen in *Tetrahymena*. Classical forward genetics has enabled significant discoveries for the last century. A critical yet time consuming step in this approach has been genetic mapping -- the identification of the causal mutation. Thanks to the development of next generation sequencing, it is now possible to quickly and cost-effectively sequence whole genome of a mutant strain in order to identify the causal mutation. This approach has been successfully applied in several genetic model organisms. Recent effort to expand such elite group of model organisms has been fruitful. *Tetrahymena* is particularly suitable for forward genetic approach thanks to its ease of genetic manipulation and large-scale rapid screening. I and colleagues developed whole genome sequencing (WGS) based pipeline to identify causal mutation with single nucleotide resolution. This pipeline has been utilized to identify causal mutations of several *Tetrahymena* mutants that were isolated nearly half a century ago [1, 2]. A variation of forward genetics is suppressor screen: a screen for additional mutations that suppress phenotypes caused by an existing mutation or condition. This expands researchers' ability to exploit a targeted biochemical process. Unlike classical genetic models, ciliates including *Tetrahymena* have two different nuclei in each cell. As mutations that confer phenotypes in the one of the nuclei is not inherited during sexual mating while exchange of stored genetic information occurs at the other nuclear. This nuclear dualism creates unique challenges for isolating genetic suppressors and subsequent identification of the casual mutation. I employed a procedure that induces "self-fertilization" and developed the first genetic suppressor screen scheme. As a proof of concept, I identified a conserved CCRK kinase as the genetic suppressor of a MOK kinase in cilia length regulation [3]. This body of work makes forward genetics drastically more accessible to researchers using *Tetrahymena* as model organism.

1. Jiang YY, Maier W, Baumeister R, Minevich G, Joachimiak E, Ruan Z, et al. The Hippo Pathway Maintains the Equatorial Division Plane in the Ciliate *Tetrahymena*. Genetics. 2017. doi: 10.1534/genetics.117.200766. PMID: 28413159.
2. Jiang YY, Maier W, Baumeister R, Joachimiak E, Ruan Z, Kannan N, et al. Two Antagonistic Hippo Signaling Circuits Set the Division Plane at the Medial Position in the Ciliate *Tetrahymena*. Genetics. 2018. Epub 2018/12/30. doi: 10.1534/genetics.118.301889. PMID: 30593491.
3. Jiang YY, Maier W, Baumeister R, Minevich G, Joachimiak E, Wloga D, et al. LF4/MOK and a CDK-related kinase regulate the number and length of cilia in *Tetrahymena*. PLoS genetics. 2019;15(7):e1008099. Epub 2019/07/25. doi: 10.1371/journal.pgen.1008099. PMID: 31339880.

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

Research Support

1. LF4 negatively regulates cilia length in *Tetrahymena thermophila*

Primary researcher. This project studied the function of LF4/MOK kinase in cilia length regulation. LF4 was shown to negatively regulates IFT speed and frequency which are closely related to the assembly of cilia. Genetic suppressor screen identified a conserved CDKR1/MAPK as the upstream activator of LF4/MOK.

2. Intracellular pattern formation in *Tetrahymena thermophila*

Primary researcher. This project aimed to identify the causal mutations of several *Tetrahymena* mutants, in which the intracellular pattern is disrupted or altered during cell division. Further investigation localized the relevant gene products. All three proteins studied have polarized localization along the A-P axis during cell

division. Initial discovery includes two antagonistic Hippo signaling circuits that control the position of the division plane. A conserved cyclin E protein was shown to balance the activity of one of the Hippo kinase. Such balance is required in initiating the formation of division boundary and positioning it at the middle of the dividing cell. Cortical exclusion emerges as the principle mechanism of regulating intracellular pattern during cell division.

Scholastic Performance

Year	Course at University of Georgia	Grade
2009	Molecular Cell Biology	A
2010	Advanced Biochemistry and Molecular Biology	B
2011	Biomedical Grant Writing	B+
2011	Advanced Cellular Biology	A
2012	Problems in Cellular Biology	A
2015	Problems in Cellular Biology	A