OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

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NAME: Melanie D. Ohi

eRA COMMONS USER NAME (credential, e.g., agency login): Ohimd1

POSITION TITLE: Associate Professor

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

| INSTITUTION AND LOCATION | DEGREE(if applicable) | Completion DateMM/YYYY | FIELD OF STUDY |
| --- | --- | --- | --- |
| Pacific Lutheran University, Tacoma, WA | BS | 1996 | Chemistry |
| Vanderbilt University Medical School, Nashville, TN | Ph.D. | 2002 | Cell Biology |
| Harvard Medical School, Boston, MA | Postdoctoral | 2007 | Cryo-Electron Microscopy |

**A. Personal Statement**

My lab uses a combination of genetics, proteomics, biochemistry, and single particle cryo-electron microscopy (cryo-EM) to study the structure and function of molecular machines that are too difficult, either due to low protein concentrations or their dynamic nature, to determine using other structural approaches. Major focuses of the lab include understanding how structural rearrangements in the spliceosome translate into function and exploring how common mutations found in MDS cancer patients affect the structural integrity and function of the spliceosome. We also have a long standing interest in studying the structure and function of bacterial virulence factors. Over the last decade we have studied structures of virulence factors found in *Helicobacter pylori* a gram negative bacteria that is one of the the leading causes of gastric cancer. Using single particle cryo-EM we have determined a high-resolution structures of the pore-forming toxin VacA in solution and are now focused on determining a structure of VacA inserted into membrane and the Type IV translocation (T4SS) machinery responsible for injecting the bacterial oncogene CagA into gastric cells. We have also purified and begun structurally characterizing the T4SS machinery from *Legionella pneumophila*, the bacteria that causes Legionnaire’s Disease. The goal of these studies is to map the molecular organization of the *L. pneumophila* T4SS and understand how its structure compares to other characterized DNA and protein translocating T4SSs.

**B. Positions and Honors**

**Positions and Employment**

1996 – 2002 Graduate Student, Dr. Kathy Gould laboratory, Vanderbilt University, Nashville, TN

2002 – 2007 Post-doctoral Fellow, Dr. Tom Walz laboratory, Harvard Medical School, Boston, MA

2007 – 2014 Assistant Professor, Vanderbilt University, Nashville, TN

2014 – 2017. Associate Professor, Vanderbilt University, Nashville, TN

2017 – pres. Research Associate Professor, Life Sciences Institute, University of Michigan

2017 – pres. Associate Professor, Dept. Cell & Developmental Biology, University of Michigan Medical School

2018 – pres. Rowena G. Matthews Collegiate Professor in the Life Sciences

**Honors and Awards**

1995 – 1996 Barry M. Goldwater Scholar

1997 – 2000 National Cancer Institute Grant T32 CA09592

2002 – 2005 Jane Coffin Childs Fellow

2002 – 2005 Agouron Institute Fellow

2005 – 2007 Charles A. King Fellow

2008 – 2013 NIH Director’s New Innovator Award

2017 Vanderbilt Chancellor Faculty Fellow

**Other Experience and Professional Membership**

2008 – pres. Member, American Society for Cell Biology

2011 – pres. Member, Biophysical Society

2011 – pres. Member, Microscopy Society of America

2010 – pres. *Ad hoc* Reviewer for the Wellcome Trust

2010 – pres. *Ad hoc* reviewer for the NIH (RO1, DP5, P01, and P41 review panels) and NSF

2016 – 2017 Expert Committee Member for the Canada Foundation for Innovation’s 2017 Innovation Fund

-Structural Biology Focus

 2017 NIH U24 review panel for National Centers in Cryo-electron Microscopy

2017 – 2021 NIH MFSC Study Section, standing member

**C. Contributions to Science**

**1. Structural analysis of molecular machines involved in bacterial pathogenesis.**

Bacterial pathogens represent an increasing threat to global health and have evolved numerous approaches to infect their hosts. Bacteria have developed powerful and diverse biological weapons to infect hosts. Examples include secretion systems for injecting bacterial DNA and proteins into host cells, toxins that alter cellular functions, and enzymes that highjack host signaling pathways. Although the methods used in pathogen-host biological warfare are as diverse as the organisms that employ them, many strategies share a common theme of relying on the function of dynamic molecular machines. While some bacterial infections can be treated with antibiotics, the evolution of drug resistance makes it imperative to find alternative therapies. Effective strategies in therapeutic design will target molecular processes that are distinct to the bacteria and important for pathogenic outcome. Finding these bacterial “Achilles heels” requires a mechanistic understanding of the molecular machines deployed by bacteria for pathogenesis.

A major focus of my research program has been to generate structure-based models of dynamic molecular machines found in infectious agents and required for pathogenesis. These include the pore-forming toxins (PFTs) TcdA, TcdB and VacA secreted by the bacterium *Clostridium difficile* and *Helicobacter pylori*, botulinum neurotoxin progenitor complexes (BoNT PCs) secreted by *Clostridium botulinum*, and the *H. pylori* CagA type IV secretion system (*cag* T4SS). These machines are responsible for generating pores in membranes (TcdA, TcdB, BoNT, and VacA) or translocating bacterial proteins into the host cells (*cag* T4SS). Using single-particle electron microscopy (EM), we have determined three-dimensional (3D) structures of soluble VacA, TcdA/TcdB, and BoNT PC, as well as providing the first structural characterization of the cag T4SS. These structures and analyses now provide an exciting starting point for understanding how soluble toxins convert into lipid-spanning pores in cell membranes and how the T4SS apparatus moves CagA from *H. pylori* into host cells. We aim to generate high resolution (sub-10Å) three-dimensional (3D) structures of PFTs in both their soluble and membrane-inserted forms, as well as, a high resolution structure of the *cag* T4SS. These structural snapshots are essential for the development of new therapeutic approaches that can block specific molecular processes of pathogenesis.

1. Frick-Cheng, A., Pyrburn,T.M., Voss, B.J., McDonald, W.H., *Ohi, M.D*.\*, Cover, T.L.\* (2016) Molecular and structural analysis of the Helicobacter pylori cag type IV secretion system core complex. *mBio.* 7(1): pii: e02001-15. PMCID: PMC4725015. \*Co-corresponding authors.

 \* Featured in F100Prime as being of special significance in its field

1. Pyburn, T.M., Foegeding, N.J., Gonzalez-Rivera, C., McDonald, N.A., Gould, K.L., Cover, T.L., *Ohi, M.D*. (2016) *Helicobacter pylori* VacA oligomerizes into hexamers on membranes and changes conformation. *Molecular Microbiology.* 102(1):22-36. PMCID: PMC5035229.

\*Cover image, October, 2016 Molecular Microbiology

1. Su, M.\*, Erwin, A.L.\*, Campbell, A.M., Pyburn, T.M., Saley, L.E., Hanks, J.M., Lacy, D.B., Akey, D.L., Cover, T.L., *Ohi, M.D.* (2019) Cryo-EM analysis of *Helicobacter pylori* VacA shows structural basis of oligomerization. JMB. 431(10): 1956-1965. \*co-first authors. PMCID: In progress.
2. Chung, J.\*, Sheedlo, M.J.\*, Campbell, A.M., Sawhney, N., Frick-Cheng, A.E., Lacy, D.B.#, Cover, T.L.#, Ohi, M.D.# (2019) Structure of the *Helicobacter pylori* cag Type IV Secretion System. eLife. In Press. \*co-first authors, #co-corresponding authors.

**2. Multifaceted approaches for studying the structure and function of spliceosomes.**

Although the human genome contains ~25,000 genes, it is estimated we make over 90,000 proteins.The disparity between our genome and proteome can be explained by the activity of the spliceosome, a macromolecular machine composed of RNAs and proteins. The spliceosome precisely removes introns from pre-mRNA to generate mature messages (mRNA), a process referred to as pre-mRNA splicing. The ~3 MDa spliceosome is composed of four snRNPs (U1, U2, U5 and U4/U6) and additional pre-mRNA splicing factors. In contrast to other cellular RNPs such as the ribosome, none of the snRNPs contain a pre-formed catalytic site, and thus the formation of an active spliceosome requires numerous RNA:RNA, RNA:protein, and protein:protein rearrangements in a series of systematically defined steps requiring the assembly and disassembly of large multi-protein complexes. *The molecular organization of RNA and proteins within the spliceosome at any stage of the splicing reaction is not known and the global conformational changes that occur during the transitions from a pre- to post-splicing complex have not been characterized.* Just as structural studies of the ribosome proved instrumental in unraveling the dynamics of protein synthesis, determining the 3D structures of spliceosomal complexes and mapping positions of individual components within these structures will be crucial for understanding how the splicing reaction is catalyzed and regulated.

The primary objective of my work is to generate a structural and mechanistic understanding of how spliceosomal components organize and transition usingendogenous splicing complexes from *Schizosaccharomyces pombe*. This represents long-term research focus that has spanned my entire scientific career, starting in graduate school and continuing into my present position as an Associate Professor at University of Michigan. We are using cryo-EM, genetics, next-generation sequencing technologies, and cutting-edge computational strategies to embark on structure/function studies to improve our understanding of how proteins and RNAs involved in pre-mRNA processing work together to form a catalytic machine. *The long-term goal of my work is to generate a series of detailed snapshots of the spliceosome as it assembles, catalyzes the splicing reaction, and disassembles.*Towards this goal we take both “top-down” and “bottom-up” approaches to study intact spliceosomes purified from cells, as well as, spliceosomal sub-complexes built from recombinant proteins.

1. *Ohi, M.D.***,** Link, A.J., Jennings, J.L., McDonald, W.H., Ren, L., and Gould, K.L. (2002). Proteomics analysis reveals stable multi-protein complexes in both fission and budding yeasts containing Myb-related Cdc5p/Cef1p, novel pre-mRNA splicing factors, and snRNAs. *Mol. Cell Biol*. **22:**2011-2024. PMCID: PMC133674
2. *Ohi, M.D.*, Ren, L., Wall, J.S., Gould, K.L., and Walz, T. (2007) Structural characterization of the fission yeast U5.U2/U6 spliceosome complex. *PNAS.* 104. 3195-200. PMCID: PMC1805518
3. Livesay, B.S., Collier, S.E., Bitton, D., Bähler, J., *Ohi, M.D.* (2013) Structural and functional characterization of the N-terminus of *Schizosaccharomyces pombe* Cwf10. *Eukaryotic Cell*. 12(11): 1472-89. PMCID: PMC3837936
4. Collier, S.E., Voehler, M., Peng, D., Ohi, R., Gould, K.L., Reiter, N.J., *Ohi, M.D.* (2014) Structural and functional insights into the N-terminus of *Schizosaccharomyces pombe* Cdc5. *Biochemistry*, 53(41): 6439-51. PMCID: PMC4204884

**3. Structural analysis of F-BAR proteins required for cytokinesis.**

In a long standing collaboration (7 years) led by Dr. Kathy Gould, the Ohi lab has participated in a series of studies that have led to a reassessment of the role of F-BAR proteins in generating membrane curvature. While general dogma has posited that the main function of F-BAR containing proteins in cells is to bend membranes, Dr. Gould’s work has shown that the essential function of at least some F-BAR domains is to serve as a scaffold between membranes and other proteins, not bend membranes. My group has contributed biophysical and structural expertise to help elucidate how *Schizosaccharomyces pombe* Cdc15 and Imp2, two F-BAR proteins involved in cytokinesis, oligomerize and interact with membranes. Analytical ultracentrifugation and single particle electron microscopy studies showed that the structural conformation of Cdc15 is directly regulated by its phosphorylation state, that Cdc15 oligomerizes both in solution and when bound to membranes, and that Imp2, but not Cdc15, tubulates membranes *in vitro*. We will continue working on these studies with the Gould lab by generating higher resolution structures of membrane-bound Cdc15 with the goal of gaining a clearer mechanistic understanding of the role of Cdc15 oligomerization in membrane binding.

1. Roberts-Galbraith, R.H., *Ohi, M.D*., R.H., Ballif, B.A., Chen, J.S., McLeod, I., McDonald, W.H., Yates, J.R., and Gould, K.L. (2010). Dephosphorylation of F-BAR Cdc15 modulates its conformation and stimulates its scaffolding activity at the cell division site. *Mol. Cell*. 39(1):86-99. PMCID: PMC2916701
2. McDonald, N.A., Vander Kooi, C.W., *Ohi, M.D.,* Gould, K.L. (2015) Linear oligomerization of the Cdc15 F-BAR domain supports membrane binding without bending to form a cytokinetic anchor. *Developmental Cell*. 35(6):725-36. PMCID: PMC4691284.
3. McDonald, N.A.#, Takizawa, Y.#, Feoktistova, A., Xu, P., *Ohi, M.D*.\*, Vander Kooi, C.W.\*, Gould, K.L.\*, (2016) The tabulation activity of a fission yeast F-BAR protein is dispensable for its function in cytokinesis. *Cell Reports*. 14(3): 543-546. PMCID: PMC4731314. #, Co-first authors; \*Co-senior authors.

**4. Understanding the organization and function of cellular macromolecular machines.**

Dynamic multimolecular assemblies perform essential and fundamental cellular functions. *Although our categorization of the number and assortment of protein interactions is increasing, we still lack knowledge about how collections of proteins are precisely assembled into macromolecular machines.* My research program is positioned to attack this formidable challenge. Increasingly detailed structures of such machines will not only lead to dramatic cell biological and mechanistic insights, but also to exciting possibilities for pharmacological intervention towards specific restorative or preventative therapies for human disease. During the course of my studies, my interest has continued to be drawn towards understanding how proteins interact and function as macromolecular machines. As a student in Dr. Kathy Gould’s lab I learned how to use *S. pombe* as a model system for functionally characterizing large complexes, such as the spliceosome. As a post-doctoral fellow in Dr. Tom Walz’s lab, I learned how to use single particle cryo-EM to determine structures of large complexes. As an independent investigator, I have developed a research program with the capability of combining the necessary research tools and methods, either within my own lab or by establishing strong multi-disciplinary collaborations, to embark on structural and functional studies of biologically interesting machines. My lab continues to take an integrative approach that allows us to address questions from biophysical, biochemical, and *in vivo* perspectives.

1. Johnson A.E., Collier S.E., *Ohi M.D.*, Gould K.L. (2012) Fission yeast Dma1 requires RING domain dimerization for its ubiquitin ligase activity and mitotic checkpoint function. *J. Biol. Chem.* **287(31):**25741-25748. PMCID: PMC3406662.
2. Folkmann, A.W., Collier, S.E., Zhan, X., *Ohi, M.D.*, Wente, S.R. (2013) Gle1 functions during mRNA export in an oligomeric complex that is altered in human disease. *Cell*. 155(3): 582-593. PMCID: PMC3855398
3. Sturgill, E.G., Das, D.K., Takizawa, Y., Shin, Y., Collier, S., *Ohi M.D.*, Hwang, W., Lang, M.J., and Ohi, R.(2014) Kinesin-12 Kif15 targets kinetochore-fibers using an intrinsic two-step mechanism. *Curr.**Biol.*, 24(19): 2307-13. PMCID: PMC4207087
4. Mittendorf, K.F.#, Marinko, J.T. #, Hampton, C.M., Key, Z, Hadziselimovic, A., Schlebach, J.P., Law, C.L., Li, J., Wright, E.R., Sanders, C.R.\*, Ohi, M.D.\* (2017) PMP22 Alters Membrane Architecture. *Science Advances*. 3(7):e1700220. #co-first authors, **\***Co-corresponding authors. PMCID: PMC5498104.

**Complete List of Published Work in PubMed:**

<http://www.ncbi.nlm.nih.gov/pubmed/?term=(Ohi-M%5BAuthor%5D)+AND+(Vanderbilt%5BAffiliation%5D+OR+Harvard%5BAffiliation%5D)>

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

**Ongoing Research Support**

5R01 GM115598-02 (Ohi, Penczek) 08/01/2015 – 05/31/2019

NIH/NIGMS

*Studies of the structural rearrangements associated with the dynamic spliceosome*

The goal of this proposal is to use single particle cryo-EM combined with complimentary genetic and computational approaches to define the conformational changes required for the transition from a pre- to post-activated spliceosome.

1R01 NS095989-01 (Sanders,C. & Ohi,M.- PI’s) 04/01/2016-03/31/2021

NIH/NINDS

*Folding, Misfolding, and Function of PMP22*

Aims are to: 1) compare and contrast the folding kinetics of different CMTD mutant forms of PMP22 in order to examine whether the specific defects that trigger PMP22 instability and terminal misfolding in the cell are the same from mutant to mutant or, alternatively, whether different CMTD mutants have distinctly different defects beyond differences in stability; and, 2) determine how the instability of CMTD mutant forms of PMP22 is sensed by endoplasmic reticulum quality control.

6R01 AI118932-02 (Cover, T. & Ohi, M.- PI’s) 04/30/2016 – 01/31/2021

NIH/NIAID (VUMC Consortium-Cover, PI)

*Type IV protein secretion in Helicobacter pylori*

The long-term goals of this research are to understand the molecular mechanisms by which *H. pylori* infection can lead to disease, and to understand the basis for variation in clinical outcomes among *H. pylori*-infected persons. The aims of the project are i) To define the molecular organization of the membrane-spanning cag T4SS core complex; ii) To determine the three-dimensional structure of the cag T4SS core complex; and (iii) To define consequences of T4SS activity in animal models of *H. pylori* infection and gastric malignancy.

R01HL144131-01 (Multi-PI: Kenworthy (Contact), Ohi) 09/30/2018-08/31/2022

NIH/NHLB

*Structural basis for caveolae assembly and function*

We are using a combination of in vivo, in vitro, biophysical, and structural approaches to understand how the structure of caveolae translates into biological function.

R01AI039657-21 (PI: Cover, co-investigator, Ohi) 04/30/2018 – 03/31/2023

NIH/NIAID

*Structure and function of the Helicobacter pylori vacuolating toxin*

The goal of this research is to use a combination of animal and structural studies to understand the function of the pore-forming toxin VacA.

**Completed Research Support**

1 DP2OD004483-01 (Ohi, Melanie) 09/30/2008-06/31/2013

NIH New Innovator’s Award

Multifaceted Approaches for Studying the Structure and Function of Spliceosomes

1 R35 GM118089-01 (Chazin) 06/01/2016–08/31/2017

NIH/NIGMS

*Structural Biology of Multi-Domain Proteins and Multi-Protein Machinery in DNA Replication and Repair*

The goal is to characterize the structural architecture and functional dynamics of multi-domain proteins including RPA and XPA, and the RPA-XPA complex, using NMR, crystallography, scattering and computational modeling. We seek structural understanding and functional validation of the interactions between XPA and RPA and their role as the central scaffold of the nucleotide excision repair (NER) pathway. We will also investigate structure-based and disease-associated mutations in XPA in cell-based assays of the repair of DNA lesions and co-localization with other NER factors.

EvansMDS000-03 (Ohi) 09/01/2015 – 08/31/2018

Edward P. Evans Foundation

*How Do SF3B1 Mutations Found in MDS Patients Alter Spliceosome Structural Organization and Function?*

The goal of this proposal is to use the genetically tractable organism, *Schizosaccharomyces pombe (Sp)*, to structurally and functionally characterize how mutations found in MDS patients compromise pre-mRNA splicing.