BIOGRAPHICAL SKETCH

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NAME: Nils G. Walter, Ph.D.

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

POSITION TITLE: Francis S. Collins Collegiate Professor of Chemistry, Biophysics, and Biological Chemistry

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
Technical University of Darmstadt, Germany	Diploma	08/1991	Chemistry/Biochemistry
Max-Planck-Institute, Göttingen, Germany	Dr. Ing.	01/1995	Chemistry/Biochemistry
Max-Planck-Institute, Göttingen, Germany	Postdoctoral	10/1995	Biophysics
University of Vermont, Burlington, VT	Postdoctoral	08/1999	Biochemistry

NOTE: The Biographical Sketch may not exceed five pages. Follow the formats and instructions below.

A. Personal Statement

The overarching goal of my group is to understand structure-dynamics-function relationships in non-coding ribonucleic acid (ncRNA) using innovative single-molecule and bulk-solution biochemical and biophysical tools, and then to adapt these ncRNAs for biomedical, bioanalytical and nanotechnological applications. My group's expertise is rooted in over 20 years of experience with non-coding RNAs (ncRNAs) that, in mammals, outnumber protein-coding genes by several-fold and are key components in a multitude of essential cellular processes, such as gene regulation, translation, and splicing. The ncRNAs on which my research focuses range from small catalytic and other highly structural ncRNAs, such as the hammerhead, hairpin, hepatitis delta virus and *glmS* ribozymes as well as riboswitches with potential use in human gene therapy and relevance to human disease, to large RNA-protein complexes, such as the ribosome, spliceosome and the RNA interference machinery. Such RNA molecules are extremely dynamic over time scales of microseconds to hours and these dynamics are integral to their biological function. To understand these dynamics we combine state-of-the-art chemical, molecular biology, and biophysical approaches. In particular, we employ fluorescence techniques to study in real-time the kinetic mechanisms of ncRNAs, in bulk solution, in live cells, and at the single-molecule level. My laboratory has particularly deep expertise in using single molecule and super-resolution fluorescence microscopy techniques to investigate the structural dynamics and intracellular pathways of ncRNAs as well as DNA nanodevices; as well as detect and count single RNA and DNA molecules in complex biofluids. In addition, I founded and direct since 2010 the Single Molecule Analysis in Real-Time (SMART) Center at the University of Michigan (http://singlemolecule.lsa.umich.edu), seeded by a $1.7Mio NSF MRI-R2 grant (PI: Walter). This unique, open-access Center enables a broad set of investigators to utilize single molecule tools for their individual projects, and synergizes with the current application. Moreover, I founded and co-direct the Center for RNA Biomedicine as a grassroots effort to synergize the RNA-related research across the University of Michigan (http://www.umichrna.org/). These research and leadership roles, together with the extraordinarily collegial environment of the University of Michigan, my many broadly interdisciplinary collaborators, my roles as the Rackham Diversity Ally in broadening the participation of diverse students in our Chemistry and Biological Chemistry graduate programs, as well as Associate Director of the UM NIGMS R25 Post-baccalaureate Research Education Program (PREP) and Co-Director of the UM NIBIB Microfluidics in Biomedical Sciences Training Program, make me well suited as a user within the current grant proposal. My current h-index is 57 (per Google Scholar) based on over 180 publications that have driven single molecule fluorescence microscopy applications in RNA biology and DNA nanotechnology for 20 years at

the University of Michigan, and even before that, starting as a graduate student with Manfred Eigen at the Max-Planck-Institute for Biophysical Chemistry in Göttingen, Germany. Never short of ideas, my innovativeness has manifested in numerous publications in *Science*, *Science Signaling*, *Nature*, *Nature Structural & Molecular Biology*, *Nature Nanotechnology*, *Nature Methods*, *Nature Communications*, *Cell*, *Molecular Cell*, the *Proceedings of the National Academy of the USA*, and many others. In the process, I have so far mentored 27 PhD students until their graduation (4 more are currently in the group) and had 25 postdoctoral fellows (12 more currently) advance their training in my group. The following four publications perhaps best highlight my scientific experience and qualifications:

1. Zhuang, X., Kim, H., Pereira, M.J.B., Babcock, H.P., **Walter, N.G.** and Chu, S. (2002) Coupling of structural dynamics and function in single ribozyme molecules. *Science* **296**, 1473.
2. Pitchiaya, S., Androsavich, J.R. and **Walter, N.G.** (2012) Intracellular single molecule microscopy reveals time and mRNA dependent microRNA assembly. *EMBO rep*. **13**, 709-715.
3. Blanco, M.R., Martin, J.S., Kahlscheuer, M.L., Krishnan, R., Abelson, J., Laederach, A. and **Walter, N.G.** (2015) Single molecule cluster analysis dissects splicing pathway conformational dynamics. *Nat. Methods* **12**, 1077-1084.
4. Johnson-Buck, A., Su, X., Giraldez, M.D., Zhao, M., Tewari, M. and **Walter, N.G.** (2015) Kinetic fingerprinting to identify and count single nucleic acids. *Nat. Biotechnol.* **33**, 730-732.

B. Positions and Honors

1989 Fellowship from the German National Merit Foundation ("Studienstiftung des deutschen Volkes")
1991 Summa cum laude Chemistry graduate of the Technical University of Darmstadt, Anton Keller Prize for best Chemistry Diploma
1992 Kekulé Ph.D. Scholarship from the Fonds of the German Chemical Industry Association
1995 Summa cum laude Ph.D. graduate, Technical University Darmstadt and the Max-Planck-Institute for Biophysical Chemistry
1995 Feodor-Lynen Postdoctoral Research Fellowship from the Alexander von Humboldt Foundation
1996 Otto-Hahn medal 1995 for Outstanding Researchers of the Max-Planck Society
1999 Assistant Professor of Chemistry
2002 Dow Corning Assistant Professorship of the University of Michigan
2004 Camille Dreyfus Teacher-Scholar Award
2005 Associate Professor of Chemistry
2006 JILA Distinguished Visitor Fellowship
2006 Alumnus of the Year Award, Sherbrooke RiboClub
2006 Visiting Scholar, Harvard University (Sunney Xie group)
2009 Professor of Chemistry
2010 Founding Director, Single Molecule Analysis in Real-Time (SMART) Center, U. of Michigan
2011 Buchanan Lecturer, Bowling Green State University
2011 Selection into the ADVANCE Program for Executive Leadership of the College of LS&A, University of Michigan
2011 Election as AAAS Fellow
2012 Alexander von Humboldt Foundation Visiting Scholar, Johann Wolfgang Goethe University Frankfurt (Harald Schwalbe group)
2013 Faculty Recognition Award, University of Michigan
2013 Imes and Moore Faculty Award, College of Literature, Science & the Arts, University of Michigan
2015 Associate Director, Michigan Post-baccalaureate Research Education Program (PREP)
2015 Co-Director, Microfluidics in Biomedical Sciences Training Program
2015 Harold R. Johnson Diversity Service Award, University of Michigan
2015 Jean Dreyfus Boissevain Lecturer 2015, Trinity University, San Antonio, TX
2016 Founding Co-Director, Center for RNA Biomedicine, U. of Michigan
2016 Professor of Biological Chemistry
2017 RNA Society Mid-Career Award 2017
2017 Francis S. Collins Professorship of Chemistry, Biophysics, and Biological Chemistry
2018 Visiting Sabbatical Scholar, Chan-Zuckerberg Biohub, San Francisco (hosted by Stephen Quake)
2018 Prasanta Datta Memorial Scholarship from the Department of Biological Chemistry, University of Michigan, for sabbatical travel

C. Contribution to Science

1. My PhD work with Nobel Laureate Manfred Eigen at the Max-Planck-Institute for Biophysical Chemistry established new forms of *in vitro* evolution as a way to endow nucleic acids with uncommon properties such as triplex formation. I particularly used non-radioactive fluorescence detection techniques for monitoring the isothermal amplification of replicating DNA molecules. In addition, I established fluorescence correlation spectroscopy as a way to detect pathogens and probe hybridization to pathogen RNA. Key publications emerging from this period include the following papers.
   a. **Walter, N.G.** and Strunk, G. (1994) Strand displacement amplification as an *in vitro* model for rolling-circle replication: Deletion formation and evolution during serial transfer. *Proc. Natl. Acad. Sci. USA* **91**, 7937-7941.
   b. **Walter, N.G.** (1995) Modelling viral evolution *in vitro* using exo$^-$ Klenow polymerase: Continuous selection of strand displacement amplified DNA that binds an oligodeoxynucleotide to form a triple-helix. *J. Mol. Biol.* **254**, 856-868.
   c. Schwille, P., Oehlenschläger, F. and **Walter, N.G.** (1996) Quantitative hybridization kinetics of DNA probes to RNA in solution followed by diffusional fluorescence correlation spectroscopy. *Biochemistry* **35**, 10182-10193.
   d. **Walter, N.G.**, Schwille, P. and Eigen, M. (1996) Fluorescence correlation analysis of probe diffusion simplifies quantitative pathogen detection by PCR. *Proc. Natl. Acad. Sci. USA* **93**, 12805-12810.

2. My postdoctoral work with John Burke at the University of Vermont addressed structure-dynamics-function relationships in RNA enzymes, particularly the hairpin ribozyme. Through a number of studies, I was able to demonstrate that fluorescence quenching and fluorescence resonance energy transfer (FRET) are able to extract the kinetics and thermodynamics of secondary and tertiary structure formation, respectively. In particular, I was able to demonstrate that the hairpin ribozyme undergoes a large-scale "docking" event that forms its catalytic core. I was also involved in seminar work that showed that the hairpin ribozyme – like other small RNA enzymes – does not require Mg^{2+} in its catalytic step. Key publications emerging from this period include the following papers.
   a. **Walter, N.G.** and Burke, J.M. (1997) Real-time monitoring of hairpin ribozyme kinetics through base-specific quenching of fluorescein-labeled substrates. *RNA* **3**, 392-404.
   b. **Walter, N.G.**, Hampel, K.J., Brown, K.M. and Burke, J.M. (1998) Tertiary structure formation in the hairpin ribozyme monitored by fluorescence resonance energy transfer. *EMBO J.* **17**, 2378-2391.
   c. Murray, J.B., Seyhan, A.A., **Walter, N.G.**, Burke, J.M. and Scott, W.G. (1998) The hammerhead, hairpin and VS ribozymes are catalytically proficient in monovalent cations alone. *Chem. Biol.* **5**, 587-595.
   d. **Walter, N.G.**, Burke, J.M. and Millar, D.P. (1999) Stability of hairpin ribozyme tertiary structure is governed by the interdomain junction. *Nat. Struct. Biol.* **6**, 544-549.

3. Since 1999 at the University of Michigan, I have consistently pioneered the development of single molecule fluorescence microscopy (SMFM) and computational approaches to dissect the mechanistic underpinnings of non-coding RNAs (ncRNAs) and DNA nanodevices first *in vitro*, then in cell extracts and now in live cells. Once considered cellular junk, ncRNAs are rapidly emerging as central, often evolutionarily conserved components of cellular phenomena that are critical to human health, including genome maintenance, regulation of gene expression, cell speciation and differentiation. To date, my most significant published achievements include the mechanistic dissection of heterogeneous folding and catalysis in the hairpin and HDV ribozymes; the discovery that the spliceosome responsible for pre-messenger RNA processing acts as a biased Brownian ratchet machine; the mechanistic probing of the intracellular RNA pathways at the single molecule level; the development of a novel detection paradigm for single RNA molecules; and the observation of single nanorobots and nanodevices through superresolution SMFM. My application of single molecule fluorescence microscopy discovered the existence of heterogeneous sub-populations in RNA enzymes that prompted many laboratories around the world to study this phenomenon. My combination of SMFM with molecular dynamics (MD) simulations allowed me to reveal the importance of long-range coupled molecular motions, water molecules and metal ions in ncRNA folding, inspiring a widespread surge of applying these tools to ncRNA. Pioneering the application of SMFM to biomachines of ever-increasing complexity, such as the spliceosome, RNA silencing machinery and engineered nanomachines, is now starting to stimulate new links between basic biology, medicine and nanotechnology, as exemplified by the University of Michigan Center for RNA Biomedicine that I co-founded. Aside from the publications above, the following key papers have emerged in the more recent past.

a. **Walter, N.G.**, Huang, C., Manzo, A.J. & Sobhy, M.A. (2008). Do-it-yourself guide: How to use the modern single molecule toolkit. *Nat. Methods* **5**, 475-489. Editorial comments in *Nat. Methods* **5** (2008) 457.
b. Lund, K., Manzo, A.J., Dabby, N., Michelotti, N., Johnson-Buck, A., Nangreave, J., Taylor, S., Pei, R., Stojanovic, M.N.*, **Walter, N.G.**, Winfree, E. and Yan, H. (2010) Molecular robots guided by prescriptive landscapes. *Nature* **465**, 206-210.
c. Krishnan, R., Blanco, M., Kahlscheuer, M., Abelson, J., Guthrie, C. and **Walter, N.G.** (2013) Biased Brownian ratcheting leads to pre-mRNA remodeling and capture prior to first-step splicing. *Nat. Struct. Mol. Biol.* **20**, 1450-1457.
d. Widom, J.R.. Nedialkov, Y.A., Rai, V., Hayes, R.L., Brooks, C.L., Artsimovitch, I. and **Walter, N.G.** (2018) Ligand-modulated cross-coupling between riboswitch folding and transcriptional pausing. *Mol. Cell* **72**, 541-552.

Complete Lists of Published Work:

http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40434435/?sort=date&direction=ascending

D. Research Support

ONGOING

NIH R01 GM062357 (Walter) 01/01/16-12/31/19
NIGMS
Riboswitch mechanism unraveled at the single molecule level
The major goal of this project is to develop biophysical techniques to dissect the mechanism of a specific translational riboswitch.

NIH 3-R01-GM-062357-14-A1-S1 (Walter) 01/01/16-12/31/19
NIGMS
Administrative supplement for instrumentation: Riboswitch mechanism unraveled at the single molecule level
The major goal of this project is to build a new single molecule fluorescence microscope in support of the parent grant.

NIH R01 GM118524 (Walter) 09/23/16-09/22/20
NIGMS
Co-transcriptional folding of single riboswitches
The major goal of this project is to develop biophysical techniques to dissect the mechanism of a specific transcriptional riboswitch.

NIH R01 GM122803-01 (Walter) 07/01/16-06/30/21
NIGMS
Timing and coordination of the conformational rearrangements mediating splicing
The major goal of this project is to develop biophysical techniques to dissect the mechanism of pre-mRNA splicing.

NIH R01 GM115857-01 (Nikonowicz) 6/1/16-3/31/20
NIGMS
Resolving structure and Mechanism of tRNA-actuated riboswitches
The major goal of this project is to mechanistically study the class of T-box riboswitches using single molecule tools.

NIH R21 CA204560-01A1 (Walter) 3/3/17-2/28/20
NCI
Single-molecule counting of cancer biomarker miRNAs in human biofluids
The major goal of this project is to optimize SiMREPS technology for the direct detection and counting of miRNA molecules in blood serum.

NIH R33 CA229023 (Tewari, Walter) 9/1/18-8/31/21
NCI
Optimization and Validation of Single-Molecule Kinetic Fingerprinting Technology for Rapid, Ultra-Specific Detection of Cancer Mutations
The major goal of this project is to optimize SiMREPS technology for the direct detection and counting of circulating tumor DNA biomarker molecules in blood serum.

NSF DMR-1607854 (Liu) 9/1/16-8/31/19
National Science Foundation
Collaborative Research: A biomimetic dynamic self-assembly system programmed using DNA nanostructures
The major goal of this build a biomimetic system resembling microtubules from DNA tiles.

RECENTLY COMPLETED MAJOR GRANTS

NIH R01 GM094450 (Chen) 7/1/17-6/30/18
NIGMS
Molecular mechanism of telomerase actions
The major goal of this project is to develop tools for studying structure-function relationships in telomerase.

Comprehensive Cancer Center/Biointerfaces Institute Research Grant (Walter, Nagrath, Ramnath)
06/01/16-05/31/18
Pilot Project-Single Molecule Characterization of Circulating Tumor Cells in Lung Cancer
The major goal of this pilot project is to analyze RNA pathways in circulating tumor cells at the single molecule level.

NIH R01 GM063162 (Wedekind) 04/01/12-03/31/16
NIGMS
Mechanism of Action of Non-Coding RNA Molecules
The major goal of this project is to determine the action of riboswitches.

Department of Defense W911NF-12-1-0420, ONR (Yan) 7/1/12-8/18/17
Army Office of Research
Translating Biochemical Pathways to Non-Cellular Environment
The major of this proposal is to use origami to spatially organize enzyme cascades and photosynthetic systems.

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: 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 Date MM/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. I hve had a long-standing interest in understanding the mechanisms of how protein and RNA organize into functional complexes. 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. The goal of these studies is to use these structures as starting points for understanding the molecular mechanisms involved in cancer formation and progression. In this proposal, we are collaborating with Dr. Nils Walter using single particle cryo-EM to understand mechanistically how riboswitches lead to translational pausing in bacterial RNA polymerase.

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.

a. 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

b. 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

c. 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.

d. 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 using endogenous 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.

a. *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

b. *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

c. 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

d. 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.

a. 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

b. 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.

c. 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.

a. 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.

b. 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

c. 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

d. 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.