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.
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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

| INSTITUTION AND LOCATION | DEGREE | Completion Date | 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 ribonucleic acids, RNAs, 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 one of the two PIs of the current grant proposal. My current h-index is 58 (per Google Scholar) based on over 185 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 28 PhD students until their graduation (9 more are currently in the group) and had 20 postdoctoral fellows (10 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. 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.
			3. 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.
			4. Pitchiaya, S., Mourao, M.D.A., Jalihal, J., Xiao, L., Jiang, X., Chinnaiyan, A.M., Schnell, S. and **Walter, N.G.** (2019) Dynamic recruitment of single RNAs to processing bodies depends on RNA functionality. *Mol. Cell* **74**, 521-533.

# 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

2018 Visiting Sabbatical Scholar, Chan-Zuckerberg Biohub, San Francisco (hosted by Stephen Quake)

# 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.
2. **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.
3. **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.
4. 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.
5. **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.
6. 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 Mg2+ in its catalytic step. Key publications emerging from this period include the following papers.
7. **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.
8. **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.
9. 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.
10. **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.
11. 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.
12. **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.
13. 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.
14. 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.
15. 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:**

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

# D. Research Support

**ONGOING**

NIH R35GM131922 (Walter) 05/01/19-04/30/24

NIGMS

*The RNA nanomachines of the gene expression machinery dissected at the single molecule level*

The major goal of this project is to apply advanced mechanistic enzymology approaches to a broad set of RNAs involved in the regulation of transcription, translation and splicing.

NIH R01 GM062357 (Walter) 01/01/16-12/31/20 (NCTX)

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) 01/01/16-12/31/20 (NCTX)

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 3-R01-GM-062357-17S1 (Walter) 04/01/19-12/31/20 (NCTX)

NIGMS

*Administrative supplement for instrumentation: Riboswitch mechanism unraveled at the single molecule level*

The major goal of this second administrative supplement is to purchase a new turnkey single molecule fluorescence microscope in support of the parent grant.

NIH R01 GM118524 (Walter) 09/23/16-08/31/20

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) 05/01/17-02/28/21

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 R33 CA229023 (Tewari, Walter) 09/13/18-08/31/21

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) 09/01/16-08/31/20

National Science Foundation

*Collaborative Research: A biomimetic dynamic self-assembly system programmed using DNA nanostructures*

The major goal of this project is to build a biomimetic system resembling microtubules from DNA tiles.

*Agreement* 12/22/17-12/21/20

Alight Sciences, LLC

*A Novel Platform for the Ultra-Sensitive Detection of Biomarkers*

The major goal of this project is to detect protein targets with SiMREPS assays and demonstrate working SiMREPS assays.

The University of Michigan – internal Biosciences Initiatives award (Walter, Ljungman)

*Center for RNA Biomedicine* 01/01/19-12/31/23

The major goal of this effort is to promote and develop cross-disciplinary collaborations on RNA across campus; leverage and promote the strengths of the U-M RNA community; and provide a central organizational structure to help recruit and develop common resources.

The University of Michigan – internal Endowment for the Basic Sciences award

EBS Partnership with the Center for RNA Biomedicine 01/01/20-12/31/24

The major goal of this partnership is to promote and support the work of the Center for RNA Biomedicine.

**RECENTLY COMPLETED MAJOR GRANTS**

NIH R21 CA204560-01A1 (Walter) 03/03/17-02/28/20

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 R01 GM115857-01 (Nikonowicz) 06/01/16-03/31/20

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.

Rogel Cancer Center 07/01/19-6/30/20

*Nano-Fingerprint-Imaging of Cancer Tissues for In Situ Single-Cell Multi-Omics*

The major goal of this project is to develop SiMREPS into an in situ detection tool to quantify cellular content.

NIH R01 GM094450 (Chen) 07/01/17-06/30/18

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.

Department of Defense W911NF-12-1-0420, ONR (Yan) 07/01/12-08/18/17

Army Office of Research

*Translating Biochemical Pathways to Non-Cellular Environment*

The major goal of this proposal is to use origami to spatially organize enzyme cascades and photosynthetic systems.