BIOGRAPHICAL SKETCH

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NAME: OLIVIA S. RISSLAND

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

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
Brown University	Sc. B.	05/2004	Biology with honors; Mathematics; Classics: Latin with honors
University of Oxford	D. Phil.	06/2008	Molecular Biology
The Whitehead Institute/MIT	Postdoctoral	08/2014	Molecular Biology

A. Personal Statement

My lab seeks to understand molecular mechanisms of gene regulation with a focus on mRNA decay and translational control. We use our insights to understand disease and development, and in the long-term, we hope to apply our discoveries to developing new RNA-based therapies. In our research, we start from our strength in RNA biology, the field in which I was trained. I began my scientific career fascinated by post-transcriptional regulation, and during my career, I have investigated different aspects of mRNA control, ranging from uridylation-mediated decay (Rissland, et al. 2007, Rissland & Norbury, 2009), to microRNA-mediated repression (Schnall-Levin*, Rissland*, et al. 2011; Nam*, Rissland*, et al. 2014; Rissland, et al. 2017) to mRNA and protein decay in embryogenesis (Wang*, Ly*, et al. 2017; Zavortink, et al. 2020). After my postdoctoral training with David Bartel (Whitehead Institute), I started my lab at the Hospital for Sick Children (SickKids), jointly appointed as an Assistant Professor at the University of Toronto. In 2017, I joined the RNA Bioscience Initiative at the University of Colorado School of Medicine as an Assistant Professor. I was attracted to this position because of the collaborative atmosphere and its strength in and commitment to RNA biology.

In my lab, we always start from our strength in RNA biology, using a combination of classical molecular biology and genetics, high-throughput sequencing, and computational approaches, and our research in *Giardia* has uncovered a treasure trove of new directions in RNA biology. For instance, through our long-term and productive collaboration with the Kieft lab (Eiler, et al. 2024, Wimberly, et al. 2022), we have uncovered differences in the *Giardia* ribosome that have implications for translation initiation and mRNA surveillance basis pathways. Our experiments then sparked our interest in 2A peptides, when we discovered, serendipitously, that they do not have robust activity in *Giardia,* leading to the natural question of “why.” Our curiosity led to this project – trying to understand how 2A peptides work, how species tropism emerges, and how 2A peptides can be harnessed as a new state-of-the art in translational programming. Our expertise in RNA biology ideally positions us to explore this new exciting area of translation.

Ongoing and recently completed projects that I would like to highlight include:
W. M. Keck Foundation Award
Rissland (PI)
7/01/2024 – 6/30/2027
Lost in Translation: how cells become protein-producing factories

R35GM128680

Rissland (PI)
08/01/2018 – 07/31/2028
Cytoplasmic mechanisms of gene regulation: intersections and coordination

NSF CAREER Award 2046136
Rissland (PI)
7/15/2021 – 6/30/2026
CAREER: Protein degradation during the maternal-to-zygotic transition

R21AI167423
Rissland (PI)
Kieft (Co-PI)
09/01/2023 – 08/31/2025
Self-cleaving peptides: Mechanisms and use in diverse eukaryotic species

Teaching, Training and Mentorship. I am committed to training, mentoring, and promoting inclusive and supportive scientific research environments. I ensure that my trainees obtain their terminal degree in a timely fashion with the skills and experiences to transition into careers in biomedical research. I am also committed to expanding diversity, creating an inclusive lab, and making science equitable. To expand diversity and inclusion, together with a colleague, I established an undergraduate intern program with a local, minority-serving institution, which will enable me to train 1–3 underrepresented minority undergraduates each year.

I have experience training students at many levels, including:

- Undergraduates: 14 in my own lab, one of whom was co-first author on a paper (Wang, et al. 2017). Three of them are now pursuing advanced biomedical degrees (Ph.D. and M.D.).
- Graduate students: 1 during my graduate career; 7 in my own lab. Three have graduated with an M.Sc., and each of those has been a co-first author on ≥1 paper (Wang, et al. 2017, Lugowski, et al. 2018a, Lugowski, et al. 2018b, Narula, et al. 2019) and a co-author on 2 additional papers. [Note that ~40% of graduate students in Canada graduate with an M.Sc., and I look forward to now helping students develop over the course of a full Ph.D.] Two of my former students are in the biomedical research workforce.
- Since coming to the University of Colorado, I have taught a first-year graduate class focused on how to read and interpret papers. For the past six years, I have also served as co-director of this course.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2022–Present Associate Professor with Tenure, Department of Biochemistry & Molecular Genetics, University of Colorado School of Medicine
2017–2022 Assistant Professor, Department of Biochemistry & Molecular Genetics, University of Colorado School of Medicine
2014–2017 Assistant Professor, Department of Molecular Genetics, University of Toronto
2014–2017 Scientist, Molecular Medicine Program, The Hospital for Sick Children Research Institute
2008–2014 Postdoctoral Fellow, The Whitehead Institute for Biomedical Research/MIT
2004–2008 Graduate Student, The Sir William Dunn School of Pathology, University of Oxford

Other Experience and Professional Memberships

2024 – present **Permanent member**, RNA Mechanisms in Cancer ACS Study Section
2023 – present **Permanent member**, Molecular Genetics NIH Study Section
2022, 2023 **Ad hoc member**, RNA Mechanisms in Cancer ACS Study Section
2021 **Ad hoc member**, K99/R00 Genetics/Developmental Biology Study Section
2020 – present **Editorial Board,** Molecular Cell
2020 – present **Editorial Board,** Cell Reports
2020 **Scientific Advisor**, Make4Covid, a Colorado-based organization to provide protective equipment to front-line workers
2020 (2X) **Ad hoc member**, NIH Cell and Molecular Biology Small Business and Commercialization Readiness Program Study Section
2019 **Ad hoc member**, NIGMS Advisory Council
2018 **Ad hoc member**, MGA NIH Study Section

2017–2021	**Chair of Membership Committee**, RNA Society
2016–2017	**Founding member and national representative** for the Association of Canadian Early Career Heath Researchers
2010 – present	**Member**, RNA Society
2009 – 11, ‘14, ’15, ‘20–‘24	**Member**, Rhodes Scholarship Selection Committee

Honors and Awards

2024	W. M. Keck Investigator
2022	Awarded Tenure
2021	NSF CAREER Award
2017	Ontario Early Researcher Award (declined)
2012 – 2014	K99 Pathway to Independence Award
2009 – 2012	NIH Ruth L. Kirschstein Postdoctoral Fellowship
2004 – 2007	Rhodes Scholarship
2004	Honors in Classics: Latin
2004	Honors in Biology
2003	Phi Beta Kappa
2003	Faculty Scholar at Brown University

C. Contributions to Science

Note: “” indicates co-authorship; “+” indicates corresponding authorship.*

1. Uridylation-mediated mRNA decay. The 3′ poly(A) tail is important for controlling the post-transcriptional fate of a transcript, and the predominant cytoplasmic mRNA decay is thought to be triggered by shortening of the poly(A) tail. However, as a graduate student with Chris Norbury at the University of Oxford, my major contribution was to show that uridylation of mRNAs also stimulates their destruction. My project began with biochemically characterizing *Schizosaccharomyces pombe* Cid1, which was originally thought to be a cytoplasmic poly(A) polymerase. I discovered that Cid1, as well as some of its human orthologs, instead functions as a terminal(U) transferase (Rissland et al. 2007). I then conceived of the plan to test the function of uridylation *in vivo* and demonstrated that uridylation triggers mRNA decapping (Rissland et al. 2009). My biochemical characterization of Cid1 provided the foundations for later structural work (Yates et al. 2012). Subsequent research has demonstrated that uridylation, mediated by Cid1-family members, is important for a wide variety of RNA regulatory pathways, including decay of microRNAs and metazoan mRNA decay.

a. **O. S. Rissland**, A. Mikulasova and C. J. Norbury. “Efficient RNA Polyuridylation by Noncanonical Poly(A) Polymerases.” *Molecular and Cellular Biology*. 27 (10): 3612–24. 2007.
b. **O. S. Rissland** and C. J. Norbury. “The Cid1 Poly(U) Polymerase.” *Biochemica et Biophysica Acta: Gene Structure and Expression: Special Issue*. 1779 (4): 286–94. 2008.
c. **O. S. Rissland** and C. J. Norbury. “Decapping is preceded by 3' uridylation in a novel pathway of bulk mRNA turnover.*” Nature Structural & Molecular Biology*. 16: 616–623. 2009.
d. L. A. Yates, S. Fleurdépine, **O. S. Rissland**, L. De Colibus, K. Harlos, C. J. Norbury and R. J. C. Gilbert. “Structural Basis for the Activity of a Cytoplasmic RNA Terminal Uridylyl Transferase.” *Nature Structural & Molecular Biology*. 19: 782–787. 2012.

2. MicroRNA-mediated repression. As a postdoctoral fellow with David Bartel (Whitehead Institute), I was broadly interested in the biology of microRNAs: how microRNAs themselves are regulated; how targets are identified; how microRNAs stimulate mRNA decay. I demonstrated that, although most microRNAs are stable, some are actively degraded, and that their destruction is important for cell-cycle progression (Rissland et al. 2011). I then characterized targets of microRNAs. Most functional microRNA sites reside within 3'UTRs, but I uncovered unusually responsive ORF targets, most of which are members of a family of zinc finger transcription factors and contain numerous ORF target sites (>10) (Schnall-Levin*, Rissland* et al 2011). I also determined how different cellular contexts affect microRNA targeting, an important issue in considering the diverse biological functions of microRNAs. I found that microRNA targeting is predominantly unaffected by cellular context, and differential targeting is most often explained by secondary effects or alternative polyadenylation (Nam*, Rissland* et al. 2014). Finally, I characterized how microRNAs and the poly(A) tail impact endogenous mRNA–protein complexes (Rissland et al. 2017). Here, my major contributions were to demonstrate that microRNAs change

the binding of translation initiation factors and to characterize DDX6 binding on a transcriptome-wide scale. I conceived and implemented each of these projects.

a. **O. S. Rissland**, S-J. Hong and D. P. Bartel. "MicroRNA Destabilization Enables Dynamic Regulation of the miR-16 Family in Response to Cell Cycle Changes." *Molecular Cell*. 43 (6): 993–1004. 2011.
b. M. Schnall-Levin*, **O. S. Rissland***, W. Johnston, N. Perrimon, D. P. Bartel and B. Berger. "Unusually Effective MicroRNA Targeting within Repeat-Rich Coding Regions of Mammalian mRNAs." *Genome Research*. 21 (9): 1395–403. 2011.
c. J. Nam*, **O. S. Rissland***, C. Jan, D. Koppstein, C. Abreu-Goodger, V. Agarwal, M. A. Yildirim, A. Rodriguez, and D. P. Bartel. "Global analyses of the effect of different cellular contexts on microRNA targeting." *Molecular Cell*. 53 (6): 1031–1043. 2014.
d. **O. S. Rissland**+, A. O. Subtelny, M. Wang, A. Lugowski, B. Nicholson, S. Sidhu, C. A. Smibert, H. D. Lipshitz, and D. P. Bartel+. "The influence of microRNAs and poly(A)-tail length on endogenous mRNA–protein complexes." *Genome Biology.* 18 (1): 211–229. 2017.

3. Protein and RNA decay during the maternal-to-zygotic transition. As an independent investigator, I have been fascinated by gene regulation during the maternal-to-zygotic transition, and my lab has used *Drosophila* as a model system. In our first study (Wang et al. 2017), we focused on understanding the role of ME31B (the DDX6 ortholog) in the *Drosophila* MZT. Our major contribution was to demonstrate that the impact of ME31B binding changes during the MZT: early in development, ME31B represses translation, but after the MZT, its binding leads to mRNA destruction. Surprisingly, we also discovered that levels of ME31B and its partners, Cup and Trailer Hitch, decrease more than 10-fold during the MZT, leading to a change in the composition of mRNA–protein complexes. In the process, we developed computational and experimental tools to enable our studies, and we have continued to characterize transcriptome dynamics during the MZT (Riemondy et al. 2023). We also recently identified the mechanism by which ME31B and its partners are degraded during the MZT. Our major contribution was to uncover a new regulatory network mediated by translational upregulation of an E2 conjugating enzyme at egg activation that then directs ME31B destruction several hours later (Zavortink, et al. 2020). More recently, we have uncovered how degradation of ME31B is mediated, determining that there are at least four layers of regulatory control restricting its degradation to the MZT (Briney et al 2024.

a. M. Wang*, M. Ly*, A. Lugowski, J. D. Laver, H. D. Lipshitz, C. A. Smibert, and **O. S. Rissland**+. "ME31B globally represses maternal mRNAs by two distinct mechanisms during the *Drosophila* maternal-to-zygotic transition." *eLife.* 6: 233–254. 2017.
b. M. Zavortink*, L. Rutt*, S. Dzitoyeva*, J. Henriksen, C. Barrington, D. Bilodeau, M. Wang, L. Chen, and **O. S. Rissland**+**.** "The E2 Marie Kondo and the CTLH E3 ligase clear deposited RNA binding proteins during the maternal-to-zygotic transition." *eLife*. 9: e53889. 2020.
c. K. Riemondy, J. Henriksen, and **O. S. Rissland**+. "Intron dynamics reveal principles of gene regulation during the maternal-to-zygotic transition." *RNA*. 29 (5): 596–608. 2023.
d. C. A. Briney*, J. C. Henriksen*, C. Lin, L. A. Jones, L. Benner, A. B. Rains, R. Gutierrez, P. R. Gafken, and **O. S. Rissland**+. "Muskelin acts as a substrate receptor of the highly regulated *Drosophila* CTLH E3 ligase during the maternal-to-zygotic transition." *bioRxiv.* 2024.

4. The relationship of translation and mRNA decay in human cells. Building off our long-standing expertise in the molecular biology of mRNA decay, we have devoted substantial effort to understanding how translation and mRNA decay connect to regulate gene expression. Previous studies in model organisms have demonstrated that codon content acts as a major determinant of mRNA stability, with the current model being that differences in codon usage change translation elongation speed and so change stability. We extended these results to human cells (Narula, et al. 2019). We have now discovered a new mechanism by which codon usage regulates gene expression: through translation initiation (Barrington, et al. 2022). Importantly, we have shown for the first time in any system that nonoptimal codons repress translation initiation, and a future direction in the lab is to understand the mechanisms by which this regulation occurs. In addition, through collaborations with the Kieft and Zenklusen labs, we have found evidence that the closed-loop model may not play as ubiquitous a role as formerly thought (Vicens, et al 2018, Adivarahan, et al. 2018). Our major contributions have been to provide direct evidence that the PABP–eIF4G interaction does not affect mRNA architecture in human cells and to suggest alternative models that can drive mRNA end-to-end communication. Our research into translation forms the basis for many of the experiments in this proposal.

a. Q. Vicens, J. S. Kieft[+], and **O. S. Rissland**[+]. "Revisiting the closed loop model and the nature of mRNA 5′–3′ communication." *Molecular Cell*. 72: 805–812. 2018.
b. S. Adivarahan, N. Livingston, B. Nicholson, S. Rahman, B. Wu, **O. S. Rissland**, and D. Zenklusen. "Spatial organization of single mRNPs at different stages along the gene expression pathway." *Molecular Cell*. 72: 727–738. 2018.
c. A. Narula, J. Ellis, J. M. Taliaferro, and **O. S. Rissland**[+]. "Coding regions affect mRNA stability in human cells." *RNA*. 25: 1751-1764. 2019.
d. C. L. Barrington, A. L. Koch, G. Galindo, E. Larkin-Gero, E. J. Morrison, S. Tisa, T. J. Stasevich, and **O. S. Rissland[+]. "**Synonymous codon usage regulates translation initiation." *Cell Reports*. 42:113413. 2023.

5. Post-transcriptional gene regulation in the distant protist *Giardia lamblia*. Motivated by a desire to understand how RNA regulation has evolved in eukarya, we have turned to *Giardia lamblia* as a model system. *Giardia* is a deeply branching eukaryote and a human pathogen. Our major contribution has been to demonstrate that many "essential" RNA regulatory pathways are not present in this distant eukaryote, raising fundamental questions about the evolution of molecular machines. We determined the structure of its 80S ribosome using cryo-EM in collaboration with the Kieft lab. We found structural evidence for changes in translation initiation and the no-go decay surveillance pathway (Eiler et al. 2020). During these experiments, we also developed an improved method for large-scale growth of *Giardia*, which will enable future biochemical and structural studies (Wimberly, et al. 2022). To understand post-transcriptional regulation, we have also mapped pre-mRNA cleavage and polyadenylation sites using long-read sequencing. In doing so, we discovered that *Giardia* uses a different poly(A) signal than other eukaryotes, and we characterized its poly(A)-tail lengths for the first time (Bilodeau et al. 2022). We subsequently characterized the evolutionary dynamics of poly(A) signals and auxiliary elements across eukaryotes (Sajek et al 2024).

a. D. R. Eiler, B. T. Wimberly, D. Y. Bilodeau, **O. S. Rissland**[+], and J. S. Kieft[+]. "The *Giardia lamblia* ribosome structure reveals divergence in translation and quality control pathways." *Structure*. 32 (4): 400–410. 2024.
b. D. Y. Bilodeau, R. M. Sheridan, B. Balan, A. R. Jex, and **O. S. Rissland**. "Precise gene models using long-read sequencing reveal a unique poly(A) signal in *Giardia lamblia*." *RNA*. 28: 668–682. 2022.
c. B. T. Wimberly[+], D. Y. Bilodeau, **O. S. Rissland**[+], and J. S. Kieft[+]. "Improved methods for large-scale cultivation of *Giardia lamblia* trophozoites in the academic laboratory." *bioRxiv*. 2022.
d. M. P. Sajek, D. Y. Bilodeau, M. A. Beer, E. Horton, Y. Miyamoto, K. B. Velle, L. Eckmann, L. Fritz-Laylin, **O. S. Rissland**[+], and N. Mukherjee[+]. "Evolutionary dynamics of polyadenylation signals and their recognition strategies in protists." In press*, Genome Research.* 2024.

Complete bibliograph is available here:
https://www.ncbi.nlm.nih.gov/myncbi/10kGU8DQ9gskx/bibliography/public/

BIOGRAPHICAL SKETCH

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NAME: Wimberly, Brian Thomas

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

POSITION TITLE: Senior Scientist

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
College of William and Mary, Williamsburg VA	BS	05/1986	Chemistry
University of California, Berkeley	PhD	09/1992	Biophysical chemistry
The Scripps Research Institute, La Jolla CA	Postdoc	08/1996	Structural biology
University of Utah, Salt Lake City UT	Postdoc	08/1999	Structural biology
MRC-LMB, Cambridge UK	Postdoc	01/2001	Structural biology

A. Personal Statement

My primary research interests are the determination of the structural basis for canonical as well as aberrant mechanisms of protein translation, with an emphasis on the roles of RNA in these processes. After adapting NMR methods to determine small non-helical ribosomal RNA structures for my doctorate, I used x-ray crystallography to determine the structure of a ribosomal protein, as well as the first published structure of a ribosomal protein bound to its ribosomal RNA binding site. I then went on to play a leading role in the determination of the structure of the entire bacterial 30S ribosomal subunit in the Ramakrishnan laboratory. This postdoctoral work introduced me to antibiotics, and my next step was to spend over ten years in the pharmaceutical industry to exploit ribosome structures for the structure-based development of new therapeutics. After a return to academia, I spent a sabbatical year in at the MRC-LMB to learn the nuts and bolts of revolutionary new cryoEM methods. This sabbatical work resulted in a landmark paper describing eleven cryoEM-derived structures of intermediates found in bacterial translation initiation. After helping Jeff Kieft build a new cryoEM facility at the University of Colorado Denver (Anschutz campus), I took on the role of cryoEM facility manager for a few years. After my return to full-time research, I have been working on studies of translation, most notably in the highly divergent human pathogen *Giardia lamblia*. I developed novel methods for dramatically scaling up the *Giardia* cultivation yields, and I played a senior role in our determination of a very high resolution (2.49 Å) cryoEM structure of the emetine-bound ribosome from *Giardia*. In summary, my experience and interests provide an excellent foundation for the present proposal for structural studies of the IRES from the *Giardia lamblia* virus.

Link to my bibliography of 40 publications, issued patents, and preprints:

https://tinyurl.com/2tym3d2e

Ongoing and recently completed NIH-funded work of relevance:

NIH R21AI167423 (PI: Rissland; I am key personnel) 9/1/23 – 8/31/25 NIH/NIAID
Self-cleaving peptides: mechanisms and use in diverse eukaryotic species

NIH R21AI149210 (co-PIs: Rissland and Wimberly) 3/1/20 - 2/28/22 NIH/NIAID
Mechanisms of protein production in the parasite *Giardia lamblia*.

B. Positions, Scientific Appointments, and Honors

2023-present	Senior Scientist, New York Structural Biology Center, New York, NY
2015-2023	Research Assistant Professor, University of Colorado Anschutz, Aurora, CO
2016-2017	CryoEM Facility Manager, University of Colorado Anschutz, Aurora, CO
2015-2016	CryoEM sabbatical, Ramakrishnan laboratory, MRC-LMB, Cambridge UK
2011-2015	Research Specialist III (Kieft laboratory), University of Colorado/HHMI, Aurora, CO
2010-2011	Distinguished Research Fellow, Rib-X Pharmaceuticals, New Haven CT
2003-2010	Associate Director, Structure-Based Drug Design, Rib-X Pharmaceuticals, New Haven CT
2002-2003	Senior Research Fellow, Rib-X Pharmaceuticals, New Haven CT
2001	Pharmacia Special Recognition Award
2001-2002	Research Scientist III, Pharmacia Corporation, Kalamazoo MI
2000	Max Perutz Prize, MRC-LMB Cambridge UK
1999-2001	Consultant, RiboTargets Ltd, Cambridge UK
1994	NIH NRSA postdoctoral fellowship, The Scripps Research Institute
1986	General Electric predoctoral award, University of California Berkeley
1982-1986	Presidential Scholar, College of William and Mary
1981	National Merit Scholar Semifinalist

C. Contributions to Science

1. Structures of ribosomal components. My first years as a scientist were focused on the determination of the three-dimensional structures of ribosomal fragments. At that time (late 1980s to mid-1990s), there were virtually no three-dimensional structures of RNA other than that of transfer RNA. In addition, there were very few structures of RNA-binding proteins or of RNA-protein complexes. Ribosomal fragments therefore were a rich source of targets that would reveal new fundamental principles of RNA structure and RNA-protein recognition. For doctoral work in the Tinoco laboratory, I used multidimensional NMR methods to determine one of the first high-resolution structures of a non-helical RNA motif. This loop E motif is both highly conserved and important for ribosome function. For postdoctoral work in the Ramakrishnan laboratory, I used x-ray crystallography to determine structures of two ribosomal components. Ribosomal protein S7 constitutes part of a functionally important tRNA-binding portion of the ribosome. I also determined the first structure of a binary ribosomal protein/ribosomal RNA complex, L11-RNA, which is the target of the thiostrepton class of antibiotics. The L11-RNA structure was patented and licensed to RiboTargets Ltd, and I consulted with RiboTargets in an effort to develop new antibacterial leads.

a. Wimberly, B.T., Varani, G., & Tinoco, I., Jr. (1993). The conformation of eukaryotic 5S ribosomal RNA loop E. Biochemistry **32**, 1078-1087. PMID: 8424938
b. Wimberly, B.T., White, S.W., & Ramakrishnan, V. (1997). The structure of ribosomal protein S7 at 1.9 Å resolution reveals a beta-hairpin motif that binds double-stranded nucleic acids. Structure **5**, 1187-1198. PMID: 9331418
c. Wimberly, B.T., Guymon, R., McCutcheon, J.P., White, S.W., & Ramakrishnan, V. (1999). A detailed view of a ribosomal active site: the structure of the L11-RNA complex. Cell **97**, 491-502. PMID: 10338213
d. Wimberly, B.T., & Ramakrishnan, V. Screening methods using the crystal structure of the ribosomal protein L11/GTPase activating region rRNA complex. US Patent 6,845,328

2. Structure of the entire bacterial 30S ribosomal subunit. As the senior postdoc in the Ramakrishnan lab, I played a leading role in the determination of the crystal structure of the entire 850,000 Da bacterial 30S

ribosomal subunit. This and other ribosomal subunit structures revolutionized the field of ribosome research by providing a much higher-resolution view of how ribosomes work, and they earned my former mentor (Venki Ramakrishnan) the 2009 Nobel prize in Chemistry.

a. Clemons, W.M., Jr.*, May, J.L.C.*, **Wimberly, B.T**.*, McCutcheon, J.P., Capel, M., & Ramakrishnan, V. (1999). Structure of a bacterial 30S ribosomal subunit at 5.5 Å resolution. Nature **400**, 833-840. PMID:10476960
b. Wimberly, B.T.*, Brodersen, D.E.*, Clemons, W.M., Jr.*, Morgan-Warren, R.J., Carter, A.P., Vonrhein, C., Hartsch, T., & Ramakrishnan, V. (2000). Structure of the 30S ribosomal subunit. Nature **407**, 327-339. PMID: 11014182
c. White, S.W., Clemons, W.M., Jr., Davies, C., Ramakrishnan, V., & **Wimberly, B.T**. (2000). Structures of ribosomal proteins: high-resolution probes of the architecture and mechanism of the ribosome. In *The ribosome: structure, function, antibiotics and cellular interactions* (eds R.A. Garrett, S.R. Douthwaite, A.Liljas, A.T. Matheson, P.B. Moore & H.F. Noller), ASM Press.
d. Carter, A.P., Clemons, W.M., Jr., Brodersen, D.E., Morgan-Warren, R.J., Hartsch, T., **Wimberly, B.T.**, & Ramakrishnan, V. (2001). Crystal structure of an initiation factor bound to the 30S ribosomal subunit. Science **291**, 498-501. PMID: 11228145

3. Structures of ribosomes bound to antibiotics. During these postdoctoral years, I also determined structures of the bacterial 30S ribosomal subunit bound to clinically important antibiotics. These antibiotic structures further spurred my interest in applied research to exploit the new ribosome structures to develop new antibiotics.

a. Carter, A.P.*, Clemons, W.M., Jr.*, Brodersen, D.E.*, Morgan-Warren, R.J., **Wimberly, B.T.****, & Ramakrishnan, V.** (2000). Functional insights from the structure of the 30S ribosomal subunit and its interaction with antibiotics. Nature **407**, 340-348. PMID: 11014183
b. Brodersen, D.E., Clemons, W.M., Jr.,Carter, A.P., Morgan-Warren, R.J., **Wimberly, B.T.**, & Ramakrishnan, V. (2000). The structural basis for the antibiotics tetracycline, pactamycin, and hygromycin B on the 30S ribosomal subunit. Cell **103**, 1143-1154. PMID: 11163189
c. Ramakrishnan, V., Brodersen, D.E., Carter, A.P., **Wimberly, B.T.**, & Clemons, W.M. Jr. Crystal structure of the 30S ribosome. US Patent 6,925,394
d. Ramakrishnan, V., **Wimberly, B.T.**, Brodersen, D.E., Carter, A.P., Clemons, W.M. Jr. Crystal structure of the 30S ribosome and its use. US Patent 7,606,670

4. Drug development based on ribosome structure. At Rib-X Pharmaceuticals, one of my primary responsibilities was to jump-start new projects by determining crystal structures of ribosomes bound to natural product inhibitors. As a side benefit, many of these structures revealed interesting new aspects of how ribosomes work. While most of this work remains unpublished as trade secret IP, some of it has been disclosed in eight issued patents from Rib-X/Melinta (including two single-inventor patents). These years in industry also gave me an understanding of the many challenges in turning inhibitors into drugs.

a. Wimberly, B.T. (2009). The use of ribosomal crystal structures in antibiotic drug design. Curr. Opin. Invest. Drugs **10**, 750-765. PMID: 19649920
b. Wimberly, B.T. Protein synthesis modulators. US Patent 8,374,794
c. Wimberly, B.T. Protein synthesis modulators. US Patent 8,428,884

5. CryoEM structures of eleven states of the bacterial 30S ribosome initiation complex. As a senior Research Specialist in the Kieft laboratory from 2011-2014, I attempted to crystallize a variety of complexes of eukaryotic ribosomes bound to viral IRES elements, with the goal of obtaining a better understanding of how viruses reprogram the ribosome. Despite considerable effort, it proved impossible to grow diffraction-quality crystals of these challenging systems. I did however gain a great deal of experience with the isolation and handling of ribosomes from many different sources. As workers in other laboratories using new cryoEM detectors and methods began to solve higher-resolution structures of IRES elements bound to ribosomes, I took advantage of an offer to spend a year learning these cryoEM methods in the laboratory of Venki Ramakrishnan at the MRC-LMB in Cambridge UK. This collaboration resulted in a milestone paper in which

eleven structural states were determined of the bacterial 30S ribosomal initiation complex using cryoEM methods.

a. Hussain, T.*, Llacer, J.T.*, **Wimberly, B.T.***, Kieft, J.S., & Ramakrishnan,V. Large-scale movements of IF3 and tRNA during bacterial translation initiation. Cell **167**, 133-144 (2016). PMID: 27662086

6. Structure of the translational machinery in the divergent human pathogen *Giardia lamblia*.
In collaboration with the Rissland and Kieft laboratories, I have solved the cryoEM structure of the divergent *Giardia lamblia* ribosome, which we have determined to very high resolution (2.49 Å) in the presence of the antibiotic emetine as well as substrate mRNA and tRNAs. Determination of this structure revealed exactly how the *Giardia* ribosome differs from canonical eukaryotic ribosomes, as well as significant new insights into the mechanism of action of emetine.

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b. **Wimberly, B.T.**, Bilodeau, D., Rissland, O.S. & Kieft, J.S. 2022. Improved methods for the large-scale cultivation of *Giardia lamblia* trophozoites in the academic laboratory. [preprint] bioRxiv doi: https://doi.org/10.1101/2022.05.12.491643