BIOGRAPHICAL SKETCH

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NAME: Pyle, Anna Marie

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POSITION TITLE: Sterling Professor, Yale University, Department of Molecular, Cellular and Developmental Biology and Department of 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
Princeton University	BA	06/1985	Chemistry, Public Policy
Columbia University	PhD	06/1990	Chemistry
University of Colorado, Boulder	postdoctoral	05/1992	RNA Biochemistry

A. Personal Statement

My research focuses on the structure and function of RNA molecules and molecular motor proteins that interact with RNA. We use a combination of experimental biochemistry, cryo-electron microscopy, crystallography and cell biology to understand architectural features and molecular recognition of large RNA molecules such as self-splicing introns, regulatory noncoding RNAs, and viral genomes. In parallel, we study RNA-dependent ATPase enzymes that are central for RNA metabolism, with a focus on those involved in human innate immunity (such as RIG-I) and viral replication (such as HCV NS3). This experimental work is complemented by computational efforts to model RNA tertiary structure and to predict it from sequence.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2023-present: Advisory Board Member and Founder, RNAConnect
2022-present: Advisory Board Member and Founder, Intron$_X$ Therapeutics
2021-present: Advisory Board Chair: Helmholtz Institute for Infection Research (HIRI), Würzburg, Germany
2021-present: Standing Committee Member: Max Planck Institute of Multidisciplinary Sciences, Germany
2020-present: Advisory Board Member and Founder, RIGImmune Therapeutics
2020-2023 Advisory Board Member, RADD Pharmaceuticals
2019-present: President (until 2021) and Past-President (until 2023), RNA Society
2019-present Advisory Board Member, Telluride Science Research Center
2018-present Sterling Professor, Yale University, Department of Molecular, Cellular and Developmental Biology and the Department of Chemistry.
2016-present Advisory Board Member, Arrakis Therapeutics
2014-2016 Chair, NIH Study Section MSFA
2013-present Member, Vice-Chair, Brookhaven National Lab Science and Technology Steering Committee
2002-present Professor, Yale University
1997-present Investigator, Howard Hughes Medical Institute
1992-2002 Assistant Professor, then Professor, Columbia Univ. Dept. of Biochemistry and Biophysics

Editorial Boards and Service

2019-present Editorial Board Member, *The RNA Journal*

2013-present Editor (with David Christianson), *Methods in Enzymology*
2007-present Editorial Board Member, *Journal of Molecular Biology*
2006-16 Contributing Editor, *Current Opinion in Structural Biology* volumes **16, 21 & 36**

Meeting Organization
2025 Co-Organizer, FASEB meeting on RNA in Cancer
2023, '04 Co-Organizer, Nucleic Acids Gordon Conference
2021 Co-Organizer, Keystone Meeting on Long Noncoding RNAs
2021 Co-Organizer, EMBL Conference: SARS-CoV-2, Two Years On
2021, '15, '98 Co-Organizer RNA Society Annual Meeting
2014, '16,'18 Co-Founder and Co-organizer, Telluride: Challenges in RNA Structural Modeling and Design
2012 Co-Organizer, Keystone Meeting on Structural Biology of Cellular Processes
2008 Co-Organizer, American Society of Biochemistry & Molecular Biology Annual Meeting
2003, '01 Co-Founder and Co-Organizer, FASEB Helicase Meeting

Honors
2023 National Academy of Sciences
2022 Lifetime Service Award RNA Society
2022 Yale Faculty Innovation Award
2021 Eli Lilly and Company Lecturer, American Society of Virology
2018 Sterling Professor of Molecular, Cellular and Developmental Biology, Yale University
2018 Jerry A. Weisbach Memorial Lecture, Rockefeller University
2018 Gomberg Lecture, University of Michigan
2018 Novartis Lecture, University of Pennsylvania
2018 Jerry A. Weisbach Memorial Lecture, Rockefeller University
2017, '18, '19 Blavatnik Fund for Innovation Award, New Haven
2016 Member, Connecticut Association of Science and Engineering
2016 Green Lectureship in Enzymology, University of Wisconsin, Madison
2013 Distinguished Lecturer, Frontiers in Chemistry, Case Western Reserve
2013 Michael Gait Award and Lectureship, Cambridge University
2011 Distinguished Visitor, European Molecular Biology Laboratory, Heidelberg
2011 Karl Friedrich Bonhoeffer Lecturer in Biophysics, Göttingen
2007 Fellow, American Association for the Advancement of Science
2005 Member, American Academy of Arts and Sciences
2002 Mayor's Award for Excellence in Science and Technology, New York

C. Contributions to Science

1. *The RIG-I family of innate immune receptors: Discovery, structural characterization, mechanistic investigation and pharmacological targeting*. During our studies on biochemical reaction mechanisms of superfamily 2 helicases (SF2 proteins, see Contribution X, below), we became involved in a collaboration with the laboratory of Paul Fisher, who had identified a SF2 protein that was fused to a set of protein domains implicated in cell death (caspase recruitment domains, or CARDs). After characterizing this curious protein together, we reported the discovery of MDA-5 (Kang et al., PNAS 2002), which was the first RIG-I-like receptor (RLR) to be identified, purified and characterized, and which was subsequently shown by others to play key roles during innate immunity, along with its two closely-related cousins RIG-I and LGP2. Lacking any biochemical or structural information on this essential group of enzymes, we set out to solve a high-resolution structure of this protein family and to determine its molecular mechanism of action. To this end, we solved the first crystal structures of an RLR protein, reporting a high-resolution structure of human RIG-I bound to double-stranded RNA. This structure and accompanying studies established that RIG-I has a unique multidomain architecture that enables it to recognize viral dsRNA and thereby trigger the innate immune cascade (Luo et al., Cell 2011). Together with simultaneous publications on RIG-I structure from the laboratories of Stephen Cusack and Joseph Marcotrigiano, a detailed picture of the RLR activation mechanism began to emerge, showing how these innate immune receptors serve as the first line of defense against viral RNA infection in mammalian cells. We have established how RIG-I differentiates host from viral RNA and signals rapidly using structural and cell biological approaches (Thoresen, MolCell

2023, Wang, MolCell 2022 and Nature Communications 2023). We went on to establish the precise molecular determinants for viral dsRNA recognition and the role of ATP, thereby showing how RIG-I becomes specifically activated by viral RNAs and avoids constitutive activation by dsRNAs in the host cytoplasm (Kohlway et al., *EMBO Reports* 2013; Rawling et al.*, eLife* 2015, Linehan et al. Science Advances 2018). This information enabled us to design potent, specific synthetic agonists and antagonists of RIG-I that are now being broadly implemented as antiviral and antitumor agents.

a. Dahai Luo, Steve C Ding, Adriana Vela, Andrew Kohlway, Brett D Lindenbach, **Anna Marie Pyle**. (2011). Structural insights into RNA recognition by RIG-I. Cell, 147(2), 409-22. PMID: 22000018
b. Melissa M Linehan, Thayne H Dickey, Emanuela S Molinari, Megan E Fitzgerald, Olga Potapova, Akiko Iwasaki, **Anna M Pyle**. (2018) A minimal RNA ligand for potent RIG-I activation in living mice. Sci Adv*,* 4(2), e1701854. PMID: 29492454
c. Daniel Thoresen, Drew Galls, Benjamin Götte, Wenshuai Wang and **Anna Marie Pyle** (2023) A rapid RIG-I signaling relay mediates efficient antiviral response. Molecular Cell, 83, 90-104. PMID: 36521492
d. Tianyang Mao, Benjamin Isrealow, Carolina Lucas, Chantal B F Vogels, Maria Luisa Gomex-Calvo, Olga Fedorova, Mallery I Breban, Bridget L Menasche, Huiping Dong, Melissa Linehan, Yale SARS-CoV-2 Genome Surveillance Initiatiive,: Craig B Wilen, Maria L Landry, Nathan D Grubaugh, **Anna M Pyle,** & Akiko Iwasaki. (2022) A stem-loop RNA RIG-I agonist protects acute and chronic SARS-CoV-2 infection in mice. J Exp Med, 219(1), e20211818. PMID: 34757384

2. *Group II intron splicing and retrotransposition: Determination of molecular structures and mechanisms.* Pre-mRNA splicing is among most important RNA processing pathways in eukaryotic metabolism and it is carried out by highly complex ribonucleoprotein machine called the spliceosome. Due to similarities in the splicing mechanism of spliceosomal RNA processing and that of group II introns, an ancient class of large ribozymes and retroelements, it had been hypothesized that the two shared a common ancestor and that group II introns might hold the key to understanding the chemical mechanism and key structural features for all of pre-mRNA splicing. With this backdrop, I started my lab with a focused effort to determine the structure and molecular mechanism of group II introns in both splicing and retrotransposition. Among our early accomplishments was the creation of multiple-turnover group II intron ribozyme constructs that enabled us to determine the mechanistic role of individual atoms within their conserved catalytic core and to begin using chemical probing technologies to obtain overall insights into their folding and structures. This lay the foundation for our efforts to solve the first high-resolution crystal structure of a group II intron (Toor et al, Science 2008), and to elucidate the role of a complex heteronuclear metal active site in the various stages of splicing (Marcia et al, Cell 2012). We used cryo-EM to resolve all of the individual steps in group II intron splicing via the branching pathway (Xu et al, Nature 2023), and we have captured the first step of DNA retrotransposition by a group II intron-RT complex (Chung et al, Science 2022). Because many group II introns encode a specialized reverse-transcriptase that facilitates both splicing and retrotransposition, we embarked on parallel studies to visualize and mechanistically characterize this "maturase protein". To this end, we solved the first high resolution crystal structure of a group II intron-encoded RT (Zhao et al, Nature Str. Mol. Biol.). We revealed that these enzymes are structurally unrelated to other RT enzymes, but they are closely related to RNA-dependent RNA polymerases and to the conserved spliceosomal protein known as Prp8. The similarities we identified between both RNA and protein components of group II introns and the spliceosome established conclusively that the two systems share a common ancestor and that the spliceosome is likely to have evolved from an ancient, disarticulated group II intron (Zhao & Pyle, Curr Op Str Biol 2017). Our structural studies on group II intron RTs resulted in the discovery of an extraordinarily processive RT that efficiently copies kilobase RNA genomes in a single pass (Guo et al. JMB 2020). This remarkable enzyme (known as MarathonRT) is now commercially available and in use by hundreds of labs to study structure and function of long RNA molecules (it was essential for our studies of SARS-CoV-2, as described below). We continue to study the Group II intron/maturase holoenzyme, as it continues to reveal unexpected insights into biology and evolution.

a. **Anna Marie Pyle.** (2016) Group II Intron Self-Splicing. Annu Rev Biophys, 45:183-205. PMID:27391926
b. Navtej Toor, Kanagalaghatta Rajashankar, Keving s Keating, & **Anna Marie Pyle**. (2008) Structural basis for exon recognition by a group II intron. Nat Struct Mol Biol, 15(11), 1221-2.PMID: 18953333

c. Chen Zhao & **Anna Marie Pyle**. (2016) Crystal structures of a group II intron maturase reveal a missing link in spliceosome evolution. Nat Struct Mol Biol, 23(6), 558-65. PMID: 27136328
d. Ling Xu, Tianshuo Liu, Kevin Chung K, **Anna Marie Pyle**, Structural insights into intron catalysis and dynamics during splicing. 2023 *Nature*, 624, 682-688.

3. *Determining the RNA structural motifs essential for the lifecycle of positive strand viruses such as SARS-CoV-2, HCV and flaviviruses*. Using a combination of biophysical and genetic methods, we have developed a pipeline for discovering regulatory RNA structures within RNA viruses, thereby laying the foundation for drug targeting of RNA structures within pathogenic viruses. This work was inspired by our previous work on the structure of group II intron RNAs, and by a new generation of tools that facilitate study of the architectural features within long RNA molecules both in-vitro and in living cells (see point 4, below). We focused our initial work on studies of riboregulatory elements within the HCV genome, as others had identified interesting genomic structures, and because HCV is a powerful system for conducting fast reverse-genetics on genomic secondary structure. This was complementary to our ongoing studies of mechanical aproteins that are encoded by this virus, especially the NS3 helicase involved in viral replication (see point 5, below). Using chemical probing and RNA sequencing methods, we generated a comprehensive secondary structural map of the entire HCV genome and we employed genetic methods to determine whether the motifs we discovered play roles during specific stages of the lifecycle, such as replication or infectivity. Significantly, we showed that functional RNA structural elements pervade the entire viral open reading frame, resulting in a structural code that is imbedded within the genetic code of HCV, thereby expanding the adaptability of compact viral genomes (Pirakatikulr et al, Molecular Cell 2016). More recently, we applied our expertise to determine the complete secondary structure of SARS-CoV-2 genomic RNA within infected cells (Huston et al, Molecular Cell 2021, Tavares et al, J. Virol. 2020). This work established that SARS-CoV-2 is the most highly structured RNA genome discovered to date, and that it is tightly folded into a network of elaborate stem-loop motifs that span the ORF and UTRs. Within this network of motifs, we discovered new riboregulatory structures and showed that they could be targeted with antisense drugs in order to disrupt viral replication. We are continuing to work on the genomic and subgenomic RNAs of SARS-CoV-2, studying their structures and roles in promoting the inflammatory disease. Studies on HCV and flaviviruses are continuing to yield new insights into viral riboregulation.

a. Nathan Pirakitikulr, Andrew Kohlway, Brett D Lindenbach, **Anna M Pyle**. (2016) The Coding Region of the HCV Genome Contains a Network of Regulatory RNA Structures. Mol Cell, 62(1): 111-20. PMID: 26924328
b. Rafael de Cesaris Araujo Tavares, Gandhar Mahadeshwar, Han Wan, Nicholas C Huston, **Anna Marie Pyle**. (2020) The global and local distribution of RNA structure throughout the SARS-CoV-2 genome. J Virol, 95(5), e02190-20. PMID: 33268519
c. Nicholas C Huston, Han Wan, Madison S Strine, Rafael de Cesaris Araujo Tavares, Craig B Wilen, **Anna Marie Pyle**. (2021) Comprehensive in vivo secondary structure of the SARS-CoV-2 genome reveals novel regulatory motifs and mechanisms. Mol Cell, 81(3): 584-598. PMID: 33444546
d. Han Wan, Rebecca L Adams, Brett D Lindenbach,**Anna Marie Pyle** (2022) The In Vivo and In Vitro Architecture of the Hepatitis C Virus RNA Genome Uncovers Functional RNA Secondary and Tertiary Structures. J Virol. 96(8):e0194621, PMID: 35353000

4. *We developed key methods and formalisms for monitoring the folding pathways of large RNA molecules*. Just as protein folding is a central process, RNA folding is of critical importance, and we have devoted considerable attention to charting the folding pathway of large, multidomain RNA molecules, starting with our studies on the self-splicing group II intron RNA (Waldsich and Pyle, Trends in Biochem. Sci. 2007). We have shown that many large RNA molecules can fold directly and faithfully to the native state through sequential processes that are often initiated and templated by the folding of the first domain that is transcribed (the RNA 5'-end) (Zhao and Pyle, Nature Chem. Biol. 2015, Somarowthu and Pyle, Molecular Cell 2015). This work has not only helped us to understand biological mechanisms for RNA folding, but it has enabled us to perform structural biology on large RNA molecules that had eluded study in the past. Our studies on RNA structural stabilization have resulted in new methods for isolating kilobase RNA molecules in their natively folded state, and for solving biologically-relevant secondary and tertiary structures (Chillon, Methods Enz. 2015). Using these methodologies, we have succeeded in solving an

abundance of group II intron RNA structures, and determining the secondary structural features of long noncoding RNA molecules and whole viral genomes.

a. **Anna Marie Pyle**, Olga Fedorova, Christina Waldsich. (2007) Folding of group II introns: a model system for large, multidomain RNAs? Trends Biochem, 32(3): 138-45. PMID: 17289393
b. Chen Zhao, Kanagalaghatta R Rajashankar, Marco Marcia, **Anna Marie Pyle**. (2015) Crystal structure of group II intron domain 1 reveals a template for RNA assembly. Nat Chem Biol, 11(12): 967-72. PMID: 26502156
c. Srinivas Somarowthu, Michal Legiewicz, Isabel Chillion, Marco Marcia, Fei Liu, **Anna Marie Pyle**. (2015) HOTAIR forms an intricate and modular secondary structure. Mol Cell, 58(2):353-61. PMID: 25866246
d. Isabel Chillon, Marco Marcia, Michael Legiewicz, Fei Lie, Srinivas Somarowthu, **Anna Marie Pyle**. (2015) Native Purification and Analysis of Long RNAs. Methods Enzymol, 558:3-37. PMDI 26068736

5. *We established the mechanistic and structural framework for translocation and unwinding by RNA helicases*. Just as molecular machines remodel DNA molecules for replication and transcription, a large family of conserved enzymes (called the DEXH/D proteins, from helicase superfamily II (SF2)) is necessary for remodeling RNA molecules in the cell. We were the first lab to conduct detailed mechanistic enzymology on this protein family, focusing initially on viral helicases NPH-II (from pox viruses) and NS3 (from hepatitis C virus and flaviviruses) (Jankowsky and Pyle, Nature 2000; Jankowsky and Pyle, Science 2001; Beran and Pyle, JBC 2007; Myong, Pyle and Ha, Science 2007; Appleby, Pyle and Somoza, JMB 2011). Our lab characterized the scope of enzymatic activities by the SF2 helicase family, showing that they function as stepwise RNA unwinding enzymes, RNA translocases, signaling proteins, and protein displacement enzymes and we used a combination of biochemical and structural approaches to understand the physical mechanism by which ATP hydrolysis is coupled to stepwise movement along RNA. Our recent work has focused on the mechanical properties of a phylogenetically-defined subclass of SF2 proteins that bind and undergo activation by double-stranded RNAs (the RIG-I like receptors, which include RIG-I, MDA5 and LGP2, as described in point 1, above). We have been working to understand how they become recognized and activated by different types of RNA molecules, and how they differentiate host from viral RNA ligands.

a. E Jankowsky, C H Gross, S Shuman, **Anna Marie Pyle**. (2000) The DExH protein NPH-II is a processive and directional motor for unwinding RNA. Nature, 403(6768):447-51. PMID: 10667799
b. E Jankowsky, C H Gross, S Shuman, **Anna Marie Pyle**. (2001) Active disruption of an RNA-protein interaction by a DExH/D RNA helicase. Science, 291(5501):121-5. PMID: 11141562
c. Dong-chul Kang, Rahul B Gopalkrishnan, Qingping Wu, Eckhard Jankowsky, **Anna Marie Pyle**, Paul B Fisher. (2002) mda-5: An interferon-inducible putative RNA helicase with double-stranded RNA-dependent ATPase activity and melanoma growth-suppressive properties. Proc Natl Acad, 99(2):637-42. PMID: 11805321
d. Sua Myong, Michael M Bruno, **Anna M Pyle**, Taekjip Ha. (2007) Spring-loaded mechanism of DNA unwinding by hepatitis C virus NS3 helicase. Science, 317(5837):513-6. PMID: 17656723