BIOGRAPHICAL SKETCH

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NAME: Li, Hong

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

POSITION TITLE: 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
SICHUAN UNIVERSITY, Chengdu, Sichuan, China	BS	06/1983	PHYSICS
THE OHIO STATE UNIVERSITY, Columbus, Ohio	MS	08/1989	PHYSICS
UNIVERSITY OF ROCHESTER, Rochester, New York	PHD	01/1994	BIOPHYSICS
BROOKHAVEN NATIONAL LABORATORY, Long Island, New York	Postdoctoral Fellow	03/1996	X-RAY CRYSTALLOGRAPHY
CALIFORNIA INSTITUTE OF TECHNOLOGY, Pasadena, California	Postdoctoral Fellow	08/1999	RNA BIOPHYSICS & BIOCHEMISTRY
UNIVERSITY OF CALIFORNIA AT BERKELEY, Berkeley, California	Sabbatical Scholar	2010-2011	CRYOELECTRON MICROSCOPY

A. Personal Statement

I am working on two significant problems: 1) how to harness diverse CRISPR-Cas systems for technological advancement and 2) how eukaryotic ribosome is maturated.

Following trainings primarily in physical sciences, I entered RNA biology when working with my mentor John Abelson, an RNA processing biochemist and co-founder of Agouron Pharmaceuticals, at Caltech where I developed an appreciation for grounding biophysical studies in biology. Since the start of my independent career at FSU, RNA biology has experienced many transformative discoveries including ribozymes (RNA enzymes), RNA interference, atomic resolution structures of large ribonucleoprotein particles such as ribosome and spliceosome, and the CRISPR-Cas immunity, all of which fuel my passion for this extraordinary field of studies.

My expertise in structural biology is recently enhanced by the one-year sabbatical training under Eva Nogales, which has enabled me to successfully tackle challenging structural biology problems previously inaccessible. I have studied more than 30 different molecules and their complexes by structural biology with 47 PDB & 11 EMDB entries that soon to reach more than 60 and 20, respectively. One of our works on CRISPR-Cas6 has created 358 citations. I hold a patent and a provisional patent on two CRISPR-Cas-based technologies. I have written 12 review articles on these subjects (7 on ribosome biogenesis and translation, 4 on CRISPR) and delivered invited lectures at conferences including Gordon Research Conferences, Ribosome Synthesis Meeting, RiboClub, The Albany Conversation, Keystone Symposium, Biophysical Society Meeting, Zing Research Conference on Nucleic Acids, RNA Society Meeting, and The CRISPR Conference and in universities around the world.

I have taken an unconventional approach to address the long-standing question about rRNA modification and seek solutions to diseases associated with modification deficiency. We created yeast strains lacking catalytic centers in snoRNPs that produce pre-ribosome and ribosome complexes that mimic or amplify the defects of these molecules in patients with autoimmune diseases, Dyskeratosis congenita (DKC) or Prader-Willi syndrome. This strategy directly assesses the structural impact of modifications on the intact enzymes as whole as opposed to on model RNAs. We have already generated an unprecedented 3D atlas of

ribosome modifications that details both local and global impacts of each modification site. This knowledge has opened doors to a series of mechanistic studies that can ultimately lead to therapeutics.

My research is thematic and in depth. Our earlier crystallographic studies of tRNA splicing endonuclease, recent work on the small nucleolar ribonucleoprotein particle (snoRNPs) and CRISPR-Cas enzymes have revealed unified principles of the ubiquitous RNA processing events and RNA-guided catalysis. The mechanistic studies have generated novel technologies in virus detection and epigenome manipulation.

While driving the research by my own expertise in structural and biophysical biology, I have collaborated and will continue to work with experts with complementary expertise to maximize the impact: Professors Gilbert (mammalian gene editing), Petrov (ribosome function and dynamics), Dennis (virus diagnosis), Yu (yeast genetics), Yang (computation), Terns and van der Oost (microbiology), Marshall (mass spectrometry) and Stagg (cryoEM).

I am generous with my time in serving the greater community of structural biology and biophysics. I serve on the Board of Southeast Regional Collaborative Access Team (SERCAT), Biophysical Society Committee of Professional Opportunities for Women (CPOW), numerous NIH and NSF study sections, editorial board of two journals, and as meeting organizers/discussion leaders and frequent reviewers for journals. I am the Director of Institute of Molecular Biophysics that comprises 10 active faculties, 5 scientific staff, 5 administrative staff, 3 core research facilities, and a vibrant graduate program (Molecular Biophysics). I am responsible for shaping and maintaining its scientific mission, research facilities, operation budget, and other tasks typically associated with a small department. Prior to becoming the Director of the Institute in 2021, I served as the Director for the Molecular Biophysics graduate program for 10 years.

I teach every semester at both undergraduate and graduate levels. Most relevantly, I have developed and taught the only X-ray crystallography graduate course at FSU. I have graduated 16 Ph.D students and 13 Honors Thesis undergraduate students. Undergraduate students have won Howard Hughes Computational Biology Fellowship, NSF research fellowship, American Cancer Society James R. Fisher award. Graduate students have won American Heart Association Pre-doctoral fellowship and ACA Margaret C. Etter Lecture award.

Ongoing projects that I would like to highlight include:

R01 GM124622 – Li (PI)
09/01/2018-08/31/2023
Structural Biology of Ribosome Biogenesis

R01 GM1013443 – Li (PI)
05/01/2022-02/28/2026
Structural Biology of RNA Processing Enzymes in Prokaryotes

Citations

a. Zhao, Y. Rai, J. and Li, H. Regulation of Translation by Ribosomal RNA Pseudouridylation. ***Science Adv.*** 2023 Aug 18;9(33). PMC10438446.

b. Sridhara, S., Goswami, H.N., Whyms, C., Dennis, J.H. and Li, H. Virus detection via programmable Type III-A CRISPR-Cas systems. ***Nature Comm.*** 2021 Sep 27;12(1):5653. PMCID: PMC8476571.

c. Das, A., Rai, J., Roth, M.O., Shu, Y., Medina, M.L., Barakat, M.R., Li, H. Coupled Catalytic States and The Role of Metal Coordination in Cas9. ***Nature Catalysis*** 2023 (in press).

d. Xue S., Wang R., Yang F., Terns R.M., Terns M.P., Zhang X., Maxwell ES., Li H. Structural basis for substrate placement by an archaeal box C/D ribonucleoprotein particle. ***Mol Cell*** 2010 Sep 24;39(6):939-49. PubMed PMID: 20864039; PubMed Central PMCID: PMC3572848.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2021 – Director, Institutor of Molecular Biophysics, FLORIDA STATE UNIVERSITY
1999 – Member, Institute of Molecular Biophysics, FLORIDA STATE UNIVERSITY
1999 – Assistant Professor, Associate Professor and Full Professor, Department of Chemistry and Biochemistry, FLORIDA STATE UNIVERSITY

Other Experience and Professional Memberships

Grant Reviewer:

2000 – 2009 Panel member, NSF Prokaryotic Genetics and Biophysics Review Panels
2010 – 2014 Study Section Member, NIH Macromolecular Structure and Function Panel C (MSFC)
2016 – 2018 *ad hoc* member, NIH: ZGM1 TRN-9(R24); Tumor Cell Biology Study Section
2016 *ad hoc* member, NIH Special emphasis panel (MIRA) R35
2018 *ad hoc* member, NIH Special emphasis panel (MIRA) ZRG1 CB-Y55
2019 *ad hoc* member, NIH Special emphasis panel (MIRA) ZRG1 CB-G55
2020 *ad hoc* member, NIH Special emphasis panel (F30, F31, F32) ZRG1 OBT-K
2019 – 2022 mail-in reviewer, NSF Molecular Biophysics panel
2021 *ad hoc* member, NIH Special emphasis panel (early career MIRA) ZRG1 CB-U(55)R
2022 *ad hoc* member, NIH Special emphasis panel (early career MIRA) ZRG1 CB-U(55)R, MSFC

Meeting Organizer and Session Chair:

2009 Session Chair, Gordon Research Conference on RNA Editing
2015 Organizer, Albany 2015: The 19th Conversation
2016 Organizer, AAAS Symposium on Discovery and Development of CRISPR Technology
2017 Organizer, SERCAT Symposium on Structural Biology
2023 Session Chair, Fusion Nucleic Acids Conference

Editorial and Advisory Boards:

2005 – 2006 Editorial Board, Journal of Biological Chemistry
2009 – 2019 Member, Advisory Board, South Eastern Region Consortium Access Team (SER-CAT)
2013 – 2018 F1000 Faculty, F1000
2017 – Associate Editor, THE CRISPR Journal
2021 – Editorial Board，Frontiers in Genome Editing

Leadership & Mentoring Activities:

2000 – Honor Thesis Adviser for *15* Florida State University Undergraduate Students
2000 – Graduate Thesis Adviser for *26* Florida State University Graduate Students
2000 – American Heart Asso. Predoctoral Fellowship Sponsor for *7* of my graduate students
2000 – Faculty Director, X-ray diffraction facility, Florida State University
2011 – 2019 Mentor, Young Scholar Program for High School Students, Florida State University
2020 Panelist, Biophysical Society “Career in Biophysics” Zoom Panel
2019 – 2022 Member, Biophysical Society Committee of Professional Opportunities for Women

Selected Lectureship:

2010 Plenary Speaker, Zing Conference on Nucleic Acids
2012 Plenary Speaker, International Conference on Riboregulation, Royal Society of Chem. Sci.
2016 Speaker, CHRIS and JOHN RNA World Symposium, UCSF

Honors

1992 William F. Neuman Award for Outstanding Graduate Student, University of Rochester
1996 National Research Fellowship Award, F32 GM18893, National Institutes of Health
2000 Scientist Development Award, American Heart Association Florida/Ohio Valley Division
2001 New Investigator Award, James and Esther King Biomedical Research Program, Florida Department of Health
2002 Young Investigator Award (declined due to conflict), American Cancer Society
2007 Developing Scholar Award (in recognition of distinction achieved in basic research and creative activity), Florida State University
2015 – 2018 Pfeiffer Family Professorship Endowment for Cancer Research, Florida State University
2018 Elected Fellow of American Association of Advancement Science

C. Contributions to Science

My contributions to science span two areas of RNA biology: CRISPR immunity and ribosome maturation.

1. CRISPR RNA Processing

In 2006, bioinformaticist Eugene Koonin at NIH published a hypothesis paper where he proposed that the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and the associated genes (*cas*) encode a system that defends bacteria or archaea against invading viruses through an RNA-mediated RNA interference process. Though we now know that the mode of interference includes DNA interference, he correctly proposed that CRISPR encodes guide RNA that target the invaders. In collaboration with Mike and Becky Terns, I used structural biology in identifying and characterizing the first CRISPR RNA processing endonuclease, Cas6 from *Pyrococcus furiosus*, thereby providing experimental support for this novel RNA-mediated immunity process. I then independently elucidated the molecular mechanisms for RNA recognition and cleavage by Cas6 and by the novel Cas7-11 (see section 2 below), which revealed a remarkable link to the mechanism of the tRNA splicing endonuclease, thereby furthering my contribution to the broader field of RNA processing.

a. Carte J, Wang R, Li H, Terns RM, Terns MP. Cas6 Is an endoribonuclease that generates guide RNAs for invader defense in prokaryotes. *Genes Dev.* 2008 Dec 15;22(24):3489-96. PubMed PMID: 19141480; PubMed Central PMCID: PMC2607076. **(*358 times cited*).**
b. Wang R., *Preamplume G.,* Terns M. P., Terns R. M. and Li H. Interaction of the Cas6 riboendonuclease with CRISPR RNAs: recognition and cleavage. *Structure* 2011 19(2): 257-264. PMID: 21300293; PMCID: PMC3154685. (underline denotes undergraduate) (***121 times cited***)
c. Shao Y. and Li H. Recognition and cleavage of a nonstructured CRISPR RNA by its processing endoribonuclease Cas6. *Structure* 2013 21(3): 385-393. PMCID: PMC3640268.
d. Shao Y., Richter H., Sun S., Sharma K., Urlaub H., Randau L., & Li H. A non-stem-loop CRISPR RNA is processed by dual binding Cas6. *Structure* 2016 PMCID: PMC4823167.

2. Multipronged CRISPR-Csm/Cmr Enzymes

The CRISPR RNA processed by Cas6 partner with a large protein complex comprised of 5-6 proteins that defends the hosts against invader viruses through an elaborative enzymatic process. This type of CRISPR-Cas effector is categorized as Type III that contains A and B subtypes, or the Csm and the Cmr complex, respectively. My laboratory contributed to initial characterizations of both Cmr and Csm activities critical for understanding their biological roles in prokaryotic immunity. Importantly, our work first identified Cmr as an RNA-guided RNA cleavage enzyme and provided evidence for Csm as an RNA-guided DNA cleavage enzyme, both of which are now known to be true that constitute their multipronged effector activities. Mechanistic studies led to creation of an effective virus detection method (MORIARTY). We recently made a breakthrough study of the novel Type III-E, Cas7-11 complex (published slightly later than Kato et al. in Cell), that both processes CRISPR RNA and degrades target RNA, furthering our contribution to RNA processing and CRISPR effector complexes.

a. Spilman M, Cocozaki A, Hale C, Shao Y, Ramia N, Terns RM, Terns MP, Li H*, Stagg S*. Structure of an RNA silencing complex of the CRISPR-Cas immune system. *Mol Cell* 2013 Oct 10;52(1):146-52. PubMed PMID: 24119404; PubMed Central PMCID: PMC3864027. (*corresponding authors)
b. Ramia N. F., Spilman M., Tang L., Shao Y., Elmore J., Hale C., Cocozaki A., Bhattacharya N., Terns R. M., Terns M. P., Li H. and Stagg S. M. Essential structural and functional roles of the Cmr4 subunit in RNA cleavage by the Cmr CRISPR-Cas complex. *Cell Rep.* 2014 9(5):1610-1617. PMCID: PMC4269474.
c. Elmore J. R., Sheppard N. F., Ramia N., Deighan T., Li H., Terns R. M. and Terns M. P. Bipartite recognition of target RNAs activates DNA cleavage by the Type III-B CRISPR-Cas system. *Genes Dev.* 2016 30(4): 447-459. PMID: 26848045; PMCID: PMC4762429.
d. Goswami, H.N., Rai, J. Das, A. and Li, H. Molecular mechanism of active Cas7-11 in processing CRISPR RNA and interfering target RNA. *Elife.* 2022 Oct 3;11:e81678**.** PMCID: PMC9629832

3. DNA Methylation-Sensitive CRISPR-Cas9

Cas9 is arguably the most widely applicable genome manipulation enzyme owing to its simplicity and programmability. We reconstituted and thoroughly characterized Cas9 from *Acidothermus cellulolyticus* (AceCas9) as the first known Cas9 with 5'-^{5m}CpC-3'-sensitivity. This discovery has opened doors for AceCas9 and other proposed new 5'-^{5m}CpG-3'-sensitive Cas9s to epigenome manipulation and detection technologies and are the basis for the pending patent "Genome Engineering Methods Using a Cytosine-Specific CAS9" (serial #15/913,444). Our unique cell survival assay enables engineering CRISPR enzymes towards desired enzymatic properties. The impact of these Cas9s, especially that sensitive to 5'-^{5m}CpG-3' is expected to be extremely high.

a. Tsui, T.K.M., Hand, T.H., *Duboy, E.C.,* & Li, H**.** The impact of DNA topology and guide length on target selection by a cytosine-specific Cas9. *ACS Synth Biol* 2017 6(6):1103-1113. PubMed PMID: 28277645; PMCID: PMC5706465. (underline denotes undergraduate)

b. Hand T.H., Das A., *Roth M.O.*, *Smith C.L.*, *Jean-Baptiste U.L.*, & Li H. Phosphate lock residues of *Acidothermus cellulolyticus* Cas9 are critical to its substrate specificity. *ACS Synth Biol*. 2018 7(12):2908-2917. PMID: 30458109; PMCID: PMC6525624. (underline denotes undergraduate)
c. Das A., Hand T. H., *Smith C. L.*, Wickline E., Zawrotny M. and Li H. The molecular basis for recognition of 5'-NNNCC-3' PAM and its methylation state by *Acidothermus cellulolyticus* Cas9. *Nature Comm.* 2020 11(1): 6346. PMID: 33311465; PMCID: PMC7733487. (underline denotes undergraduate)
d. Roth, M.O.,and Li H. "X" marks the spot: Mining the gold in CasX for gene editing. *Mol Cell* 2022 March 17; 82(6): 1083–1085 PMCID: PMC9060429

4. SnoRNA-guided Ribosome Biogenesis

My laboratory elucidated the structural mechanisms of the archaeal homologs of both types of snoRNPs and their biogenesis chaperone R2TP. The box C/D snoRNPs add methyl group while the box H/ACA snoRNPs isomerize uridine at ~100 rRNA sites, respectively. Several special members of snoRNPs (such as U3, U14, snR30) facilitate cleavage of the precursor rRNA. Our structure and function studies simplified the seemingly complex topological relationship between snoRNPs and rRNA by revealing a one-sided rRNA binding model that allows snoRNPs to repeatedly bind and release the rRNA.

In the past two years, we initiated a unique approach to address the physiological significance of snoRNPs by systematically removing each type of modifications followed by direct examination of pre-ribosome and ribosome structures by cryoEM. High resolution structures of the strategically designed functional complexes have and will continue to create a comprehensive 3D atlas of ribosome modification that documents both detailed and global structural roles of each modification in ribosome and pre-ribosome function. Initial and less complete studies than those documented in the proposal have been published (a & b). We expect, upon completion, our work will be unique and highly impactful to the ribosome and the broader RNA modification community.

a. Zhao Y., Rai, J., Yu H., and Li H. CryoEM structures of pseudouridine-free ribosome suggest impacts of chemical modifications on ribosome conformations. *Structure* 2022 30, 1–10. PMID: 35489333
b. Zhao Y., Rai, J., Yu H., and Li H. Artificial Intelligence-assisted Structure Determination of Bfr2-Lcp5 on Pih1-depleted 90S. *Communications Biology* 2022 June 1, DOI: 10.1038/s42003-022-03500-y
c. Liang, B., Zhou, J., Kahen, E., Terns, R.M., Terns, M. P. and Li, H. Structure of a functional ribonucleoprotein pseudouridine synthase bound to a substrate RNA. *Nat Struct Mol Biol* 2009 **16**, 740-6. PMCID: PMC5706466
d. Tian S., Yu G., He H., Zhao Y., Liu P., Marshall A. G., Demeler B., Stagg S. M. and Li H. Pih1p-Tah1p puts a lid on hexameric AAA+ ATPases Rvb1/2p. *Structure* 2017 25(10): 1519-1529. PMID: 28919439; PMCID: PMC6625358.

5. Transfer RNA Splicing

Intervening sequences (introns) are found in transfer RNA (tRNA) of all three kingdoms of life and must be removed for protein synthesis. While bacteria tRNA introns are removed via self-splicing introns, archaeal and eukaryal tRNA introns are removed by a suite of enzymes that include an endoribonuclease, a ligase, and in some cases, a phosphate transferase. With John Abelson and graduate student Chris Trotta, I initiated structural studies of the splicing endonuclease and elucidated a remarkable mechanism of splice site recognition and the chemical basis for catalysis that enable an RNA structure-dependent recognition and cleavage. My structural work filled the gap in understanding tRNA splicing. My work became highly cited and has been adopted by biochemistry textbooks for teaching the subject of tRNA splicing.

a. Li H, Trotta CR, Abelson J. Crystal structure and evolution of a transfer RNA splicing enzyme. *Science* 1998 Apr 10;280(5361):279-84. PubMed PMID: 9535656.
b. Abelson, J., Trotta, C.R. & Li, H. tRNA splicing. *J Biol Chem* **273**, 12685-8 (1998). PMID: 9582290. (***208 times cited***)
c. Xue S, Calvin K, Li H. RNA recognition and cleavage by a splicing endonuclease. *Science.* 2006 May 12;312(5775):906-10. PubMed PMID: 16690865.
d. Trotta CR, Paushkin SV, Patel M, Li H, Peltz SW. Cleavage of pre-tRNAs by the splicing endonuclease requires a composite active site. *Nature.* 2006 May 18;441(7091):375-7. PubMed PMID: 16710424.

Complete List of Published Work in MyBibliography (total 77):
https://www.ncbi.nlm.nih.gov/myncbi/hong.li.1/bibliography/public/