General User Proposals

Example GUP1 review for reference

These proposals are for GUP1 (Krios access). In this early access phase we have limited availability to provide 1, 2 or 3 days Krios sessions on our existing infrastructure.

1) Project ID: NCCAT-GUP1-HJ181025 (formerly NCCAT-GUP1-HJ180927) Project Name: Structural Studies on Translational Machinery
Primary User Name: Hong Jin
eRA Commons User Name: HongJin
Institution: University of Illinois at Urbana-Champaign
Re-Submission Date: October 25, 2018

Averaged URC scores:

(i) scientific impact: **3**
(ii) technical feasibility: **1.7**
(iii) resources requested: **2.3**
(iv) user EM background and history: **3.3**
(v) geographical demographics or need: **1**
Raw average score: 2.3

Comments:

Reviewer 1:

Concern: Lacks information on number and types of different samples to be taken to NCCAT ("ribosome and its protein and RNA ligands" could be a very large number). Apart from (V), the proposal text has not changed despite previous comments to user.
Improvement: Figures changed to include cryo-EM images and class averages.

Reviewer 2:

Additional preliminary data showing cryo sample shows that the sample quality is good. It is unclear to me why a week of time is required. In my experience, this length or time seems excessive for one sample, unless initial data has been worked up to show clear discreet conformations that are limited in resolution by particle number. Therefore unless this is already known, I would suggest 2 day allocation, during which ~2500-3000 stacks at least can be collected, and workup of these data would determine whether additional time is required, which can be allocated if necessary.

Reviewer 3:

The proposal would greatly benefit if details on the protein(s) and mechanism to be studied were included in the proposal text. In addition, as a minimum the cryo-EM grid preparation strategy needs to be outlined as negative stain images are shown and Titan Krios time is requested (e.g. Where will the cryo-EM grids be prepared? Will grid preparation strategy from a previous sample translate easily to this sample?).

Project ID: NCCAT-GUP1-HJ181025
Primary User Name: Hong Jin
eRA Commons user name: HongJin
Project Name: Structural Studies on Translational Machinery

Abstract:
Protein synthesis takes place in the ribosome and is fundamental to all living cells. It directly influences important cellular processes such as development, differentiation, growth, cell fitness and cellular adaptive responses to the external environment. Consequently, regulatory dysfunction in protein synthesis underpins various human diseases including metabolic disorders, neuronal degenerative diseases and cancer. Our goal is to elucidate molecular mechanisms of translation and translational control in gene expression, and how errors in the translation process relate to human disease. Towards this goal, the overall objective of the proposed research is to elucidate the molecular determinants and mechanisms that control ribosome functions in protein synthesis and its quality control in gene expression. We use biochemistry and single-particle cryoEM to study molecular interactions of the ribosome with its protein and RNA ligands at different steps of the translation. Our research is expected to provide a molecular understanding of highly conserved protein-RNA interactions in the ribosome that contribute to the precise control of this cellular machine's function. We also hope to reveal novel ribosome functions in regulating gene expression by decipher its three-dimensional structure.

Scientific Impact:
Understanding the ribosome structure and function is an essential step for understanding gene expression and regulation. Ribosome, the translation machinery in every living cell, carries out important function of translating all the genetic information encoded in the messenger RNAs into proteins. Achievement of our experimental goals is expected to reveal previously unknown molecular interactions essential for life and contribute to establishing fundamental principles of translation in gene expression.

Scientific Feasibility:
Ribosomal complexes have proven to be a fantastic subject for the single-particle cryoEM study from the onset of this method initiated by Joachim Frank and colleagues. Over the past a few years, we witness the huge success of using single-particle cryoEM to reveal atomic structures of ribosomal complexes in translation. Today the key to success is to isolate and purify a functional complex subject to the cryoEM study. We have expertise in purifying functional translation apparatus and also a strong record in elucidating mechanisms and functions of the ribosome in each stage of translation. Each and every sample that we send to the NCCAT will be well-characterized biochemically in our lab to ensure the success of the data collection and structural determination.

Technical Feasibility:
We have a strong record in handling challenging yet high-impact projects, expertise in purifying and charactering ribosomal complexes. Trained as an NMR spectroscopist and X-ray crystallographer, the PI has gained experience in cryoEM data collection, process and 3D reconstruction during these two years. Our recent publication on the ribosomal complex structure in Nature demonstrated our capability in the field.

Resources Requested:
In order to stay competitive and obtain high-resolution structures, we critically in need of the access to a high-end cryoEM facility such as Titan Krios instruments with a Gatan K2 direct-electron detector housed at the NCCAT. Furthermore, NCCAT is the best national cryoEM resource in the North America with an easy access for us in the east coast. Since we aim for an atomic resolution for the translational apparatus, we estimate that one-week instrument time at the NCCAT is critical for our success in obtaining quality data.

Geographic/Demographics:
UIUC does not currently have cryoEM facility. We are in the process of a campus-wide discussion in obtaining medium-range cryoEM instrumentation. Our lab has access to the cryoEM instrumentation that is made available at the Northwestern University.

Quality of our sample to be submitted to the NCCAT

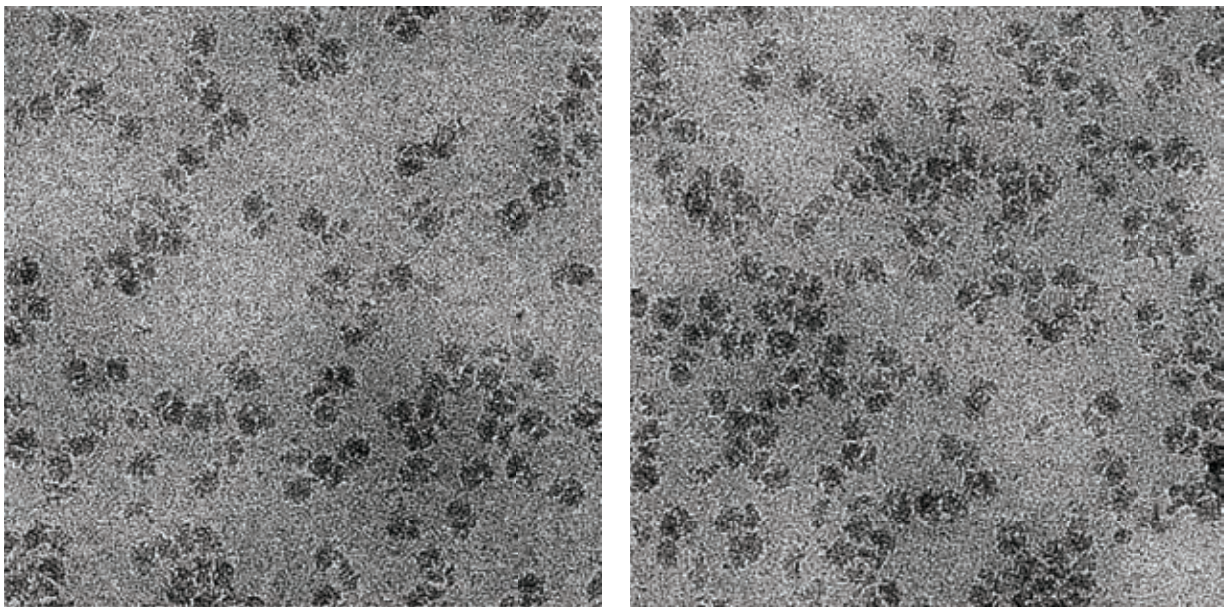


Figure 1. Representative images of ribosomal complex sample in vitrified ice.

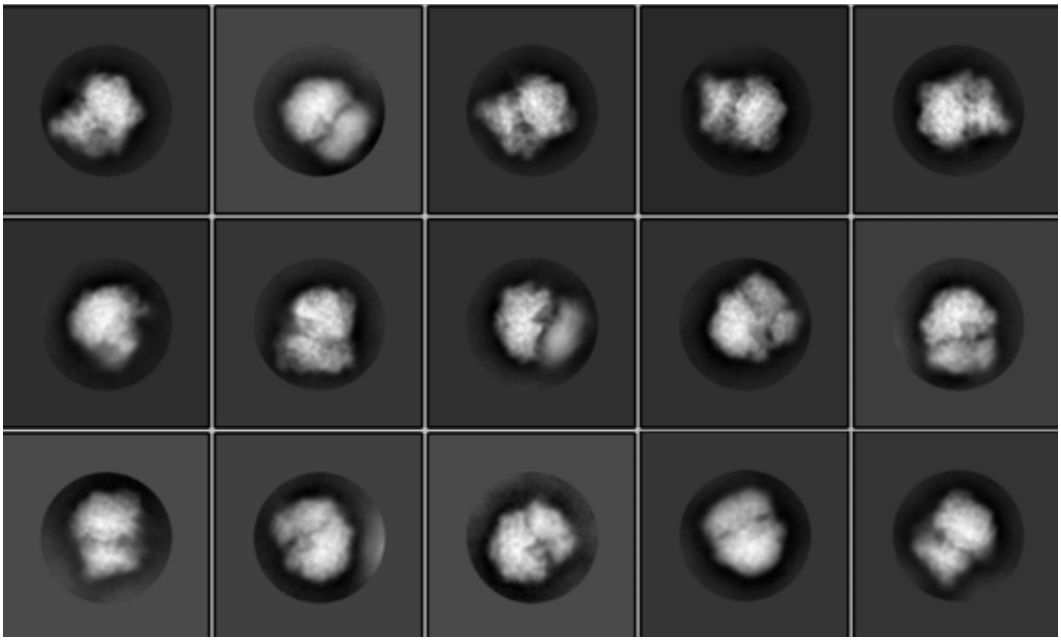


Figure 2. 2D classification of 20K particles

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: Hong Jin

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

POSITION TITLE: Assistant Professor of Biochemistry

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
Central China Normal University, Wuhan, Hubei, China	B.S.	05/1995	Chemistry
Wuhan University, Wuhan, Hubei, China	M. S.	05/1998	Analytical Chemistry
University of Massachusetts at Boston, Boston, MA		05/2000	Physical Chemistry
Yale University, New Haven, CT, USA	Ph.D.	05/2007	Biophysics
Medical Research Council (MRC) - Laboratory of Molecular biology, Cambridge, UK	Postdoctoral training	01/2008-12/2012	Biophysics, X-ray Crystallography

A. Personal Statement

My research focuses on understanding the molecular mechanisms of translation and translational regulation in the cell. My group is best known for revealing structures and functions of the ribosome, as well as RNAs and RNA-binding proteins involved in the cellular translation process that is fundamental to gene expression and regulation. I have broad expertise in Biochemistry, Biophysics and Structural Biology including macromolecule X-ray crystallography, solution NMR spectroscopy and single particle cryoEM. As a Ruth L. Kirschstein National Research Postdoctoral Fellow, I carried out extensive biochemical investigations in translation and solved several high-resolution crystal structures of ribosomal complexes with protein release factors bound along the translational termination pathway by X-ray crystallography. These include the first high-resolution crystal structure of a translational GTPase, the class II release factor RF3, bound to the hybrid state of the ribosome. These structures have addressed fundamental questions in molecular biology that have persisted since the genetic code was discovered by revealing universally conserved protein-RNA interactions that contribute to the specificity of stop-codon recognition and the catalysis of peptide release on the ribosome. I also characterized the molecular features of the ratcheting ribosome and demonstrated how a GTPase induces and stabilizes the ribosome in the hybrid state. These features are fundamental to the ribosome function in each of its essential stages of translation including initiation, elongation, termination and recycling. As a Ph.D student at Yale University, I initiated a biochemical investigation of eukaryotic small nucleolar RNA (snoRNA) and ribosomal RNA (rRNA) interactions, and solved the first structure of a snoRNA-rRNA complex by solution NMR spectroscopy. My dissertation work revealed a new RNA-RNA interaction motif, named as the Ω motif, which provided mechanistic insights into how a specific nucleotide in the rRNA is selected for modifications that are essential for cellular functions. As a young investigator, I have laid the groundwork for our research by not only developing effective experimental strategies and protocols, but also providing leadership and administrative skills. Our key findings were published in the peer-reviewed journals including *Nature, PNAS, RNA* and *Scientific Reports*. I believe my group is uniquely positioned to investigate the fundamental questions that require a combination of calculated risk-taking and a focused desire to tackle important yet underexplored areas of science. My scientific training, experience and past success in RNA and translation will certainly be of great value in pioneering our research and I look forward to the challenges and opportunities that confront our investigations.

B. Positions and Honors

Research and Professional Positions

01/2008 – 12/2012 **Postdoctoral Fellow (Supervisor: Dr. Venki Ramakrishnan)**
MRC Laboratory of Molecular Biology, Cambridge, UK

09/2009 – 12/2012 **Research Fellow in Science (Equivalent to a Faculty Position in the US)**
University of Cambridge, Lucy Cavendish College, Cambridge, UK

12/2012 – Present **Assistant Professor of Biochemistry**
Department of Biochemistry, Center for Biophysics and Quantitative Biology
University of Illinois at Urbana-Champaign

Academic and Professional Honors

2009-2012 **NIH Ruth L. Kirschstein National Research Service Award, USA**
- The award includes my postdoctoral salary and institutional allowance that covers my conference travel and part of the research expense.

2009-2012 **Ethel Cruickshank Research Fellowship, University of Cambridge - Lucy Cavendish College, UK**
- The award is equivalent to a faculty position in the US, which includes participating and providing various academic services such as seminars, lectures, governing body meetings and other administrative services. It also includes lively participation in the college life and activities in the Lucy Cavendish College and other colleges in the University of Cambridge.

2002 **Yale University Extraordinary Teaching Assistant Award**
- The award is based upon student evaluations and nominations

1992-1995 **Central China Normal University Scholarship for Academic Excellence**
- Top 2% of the university

Memberships in professional societies:

2008-Present The RNA Society, USA
2009-Present The Biophysical Society, USA
2007-Present The American Chemical Society, USA

Other Experience and Professional Activity

2007- Present Invited speaker at 16 institutions and conferences
2016 Ad Hoc reviewer for NSF (Systems and Synthetic Biology)
2016 Ad Hoc reviewer for NIH as an early stage investigator (GM-MSFC)

C. Contribution to Science

1. An induced-fit mechanism for co-translational quality control process in the ribosome

Intervening protein synthesis when an error occurs is an essential quality control mechanism that contributes to the overall accuracy and fidelity of translation in living cells. In this process, cellular signals that initiate quality control process are first deciphered in the translating ribosome, and then release factors or release factor-like proteins are recruited to terminate protein synthesis, followed by recycling of ribosomal subunits and degradation of faulty translational components. Using ArfA and RF2 as a model system, we determined the kinetics of ArfA/RF2-mediated co-translational quality control on bacterial ribosome follows an induced-fit mechanism. Since the signals that intervention is required originate in the decoding center of the small ribosomal subunit while the actions that result in termination of protein synthesis occur in the large subunit of the ribosome, *the induced-fit mechanism for co-translational quality control that we have proposed is likely to be universally conserved.*

Our results have a broader impact towards uncovering new functions of the ribosome in quality control pathways, demonstrating that beyond making proteins in living cells, ribosomes actively maintain translation accuracy and fidelity by recruiting proteins other than the canonical translational factors.

My role in this work is the PI. I designed experiments, and performed most of the experiments with a postdoctoral fellow in my laboratory in this research.

- Fuxing Zeng and **Hong Jin**
  Peptide release promoted by methylated RF2 and ArfA in nonstop translation is achieved by an induced-fit mechanism ***RNA,*** 22(1): 49–60, 2016 PMCID: PMC4691834

- Fuxing Zeng, Yanbo Chen, Jonathan Remis, Mrinal Shekhar, James C. Phillips, Emad Tajkhorshid and **Hong Jin**.
  Structural basis of co-translational quality control by ArfA and RF2 bound to ribosome. ***Nature,*** 541(7638), 554-557, 2017 PMID: 28077875

2. A dual role of the decapping activator in the eukaryotic cell.

Decapping activators facilitate removal of the 5' m^7G caps from eukaryotic mRNAs by assisting the assembly of decapping complexes or stimulating activities of the decapping enzyme, and they are known to be translational repressors to inhibit translation and promote storage or degradation of mRNAs in cytoplasm. Using biochemistry, we showed that one decapping enhancer, Sbp1, selectively promotes the translation of mRNA encoding the polyadenosine-binding protein (Pab1) and other mRNAs possessing cap-independent translation initiation activities. We further demonstrated molecular interactions important for Sbp1-specific translational regulation and the underlying molecular mechanism.

Our results not only *reveal a dual role of the decapping activator in regulating mRNA translation: a general translation repressor and a translation activator for subset of mRNAs in the cell, but also connect the two seemingly unrelated processes, decapping and translation activation of cellular mRNAs, thereby identifying a new layer of translational control in eukaryotic cells.*

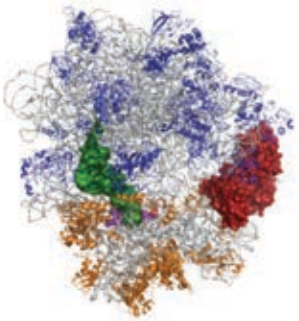
My role in this work is PI. I assembled a strong research team, designed experiments and participated the experimental work with my team.

- Alberto Brandariz-Núñez, Fuxing Zeng, Quan Ngoc Lam and **Hong Jin.**

  Sbp1 modulates the translation of Pab1 mRNA in a poly(A)- and RGG-dependent manner, ***RNA,*** *24*, 43-55, 2018 PMID: 28986506

3. Solve the first high-resolution crystal structure of a translational GTPase bound to the hybrid state of the ribosome by X-ray crystallography

Translational GTPases ensure the speed, fidelity and accuracy in each step of the translation. Ribosomes recruit the translational GTPases in the hybrid ligand-binding states where the two ribosomal subunits rotate relative to one another. Following a thorough biochemical characterization of the translation system, *I solved the first high-resolution crystal structure of a translational GTPase, the class II release factor 3 (RF3), bound to the hybrid state of the ribosome by X-ray crystallography.* The structure revealed the molecular interactions on how a GTPase induces and stabilizes the hybrid state of the ribosome and shed light on the function of RF3 in translational termination. My work also elucidated the structural features of the ratcheting ribosome in the hybrid ligand-binding state that are the fundamental feature of the ribosome function in the essential stages of translation including initiation, translocation, termination and recycling.



Furthermore, the new crystal form that I discovered has paved the way for solving new structures of ribosomes with other translational GTPases bound. These structures have opened a new field for functional investigations and have provided enormous molecular details and framework for designing new antibiotics targeting bacterial ribosome functions.

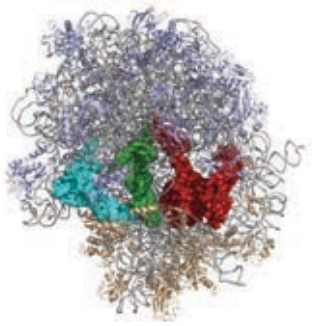
I led this project as a postdoctoral fellow. I designed and performed all the experimental work while one technician in the lab helped on a protein purification step.

- **Hong Jin**, Ann C. Kelley and V. Ramakrishnan
  Crystal structure of the hybrid state of ribosome in complex with the GTPase release factor 3.

Proceedings of the National Academy of Sciences, 108(38): 15798-15803, 2011
PMCID: PMC3179103

4. Solve the first high-resolution crystal structure of a protein release factor bound to the 70S ribosome

The specificity of stop-codon recognition and the catalytic mechanism of peptide release have been the fundamental questions in molecular biology since the elucidation of the genetic code. I achieved the major breakthrough in structural studies on ribosomal complexes in the first year of my postdoctoral training with Dr. Venki Ramakrishnan. I established a new and reproducible condition for obtaining high-resolution 70S ribosome crystals with protein factors bound and *determined the first high-resolution crystal structure of release factor 2 (RF2) bound to the 70S ribosome.* The structure revealed universally conserved protein-RNA interactions in the ribosome during termination. I also obtained structures of other ribosomal complexes along the termination pathway. Together, these structures answered long-standing questions about the specificity of stop-codon recognition and addressed the catalytic mechanism of peptide release.



Furthermore, the condition that I have established for 70S ribosome crystallization and cryo-protection has paved way for the subsequent success on obtaining structures of other bacterial ribosomal complexes in the Ramakrishnan laboratory.

I led this project to the successful completion as a postdoctoral fellow. I designed the sample preparation and crystallization plan. I performed the experiments and screened the crystals with help from my colleagues.

- Albert Weixlbaumer*, **Hong Jin***, Cajetan Neubauer, Rebecca M. Voorhees, Sabine Petry, Ann C. Kelley and V. Ramakrishnan **(*These two authors contributed equally to the work)**
  Insights into translational termination from the structure of RF2 bound to the ribosome.
  Science, 322(5903): 953-956, 2008 PMCID: PMC2642913

- **Hong Jin**, Ann C. Kelley, David Loakes, and V. Ramakrishnan
  The structure of the 70S ribosome bound to RF2 and a substrate analog provides insights into catalysis of peptide release**.**
  Proceedings of the National Academy of Sciences, 107(19): 8593-8598, 2010
  PMCID: PMC2889298

5. Solve the first structure of the human U65 snoRNA bound to rRNA by solution NMR spectroscopy

Noncoding RNAs play essential and diverse roles in regulating gene expression. My Ph.D. work focused on one type of the noncoding RNAs in eukaryotic cells, small nucleolar RNAs (snoRNAs). Base pairings between snoRNAs and sequences in eukaryotic rRNAs target specific nucleotides for modifications. Most of the modifications are clustered in the functionally important regions of the ribosome. rRNA modifications are critical for ribosome biogenesis, assembly, structure and translating function. snoRNAs play other essential functions in the cell, including participating in pre-mRNA processing, directing alternative splicing and acting as microRNA precursors. As a Ph.D. student, I developed a model system, used extensive biochemical and biophysical methods including chemical and enzymatic footprinting, native gel binding assay, electrospray mass spectrometry, and analytical ultracentrifugation experiment, to characterize the model and parent systems, and subsequently *determined the first structure of the human U65 snoRNA with and without substrate bound by solution NMR spectroscopy*. This structure revealed *a new Ω-shaped RNA interaction motif* that is conserved in all box H/ACA snoRNP-substrate complexes and provided the first physical evidence to support unique features of the substrate-recognition by box H/ACA snoRNA.

My role in this work is a Ph.D. student. I designed and carried out all the experimental investigations in this research. My Ph.D. research provided *the first structural basis for box H/ACA snoRNA-mediated pseudouridylation in eukaryotic cells*.

- **Hong Jin**, J. Patrick Loria and Peter B. Moore.
  Solution structure of an rRNA substrate bound to the pseudouridylation pocket of a box H/ACA snoRNA.
  Molecular Cell, 26(2): 205-215, 2007 PMID: 17466623

Other publications in Chemistry and Analytical Chemistry fields are:

- Fuxing Zeng and **Hong Jin**.
  Conformation of methylated GGQ in the Peptidyl Transferase Center during Translation Termination.
  Scientific Reports, *8*, 2349, 2018. PMID: 29403017
- Hong Wang, Yuan-yuan Zhao, **Hong Jin**, and Hua-shan Zhang
  N-hydroxy-succinimidyl-α-naphthylacetata as a derivatizing reagent for amino acids and oligopeptides in RP-HPLC.
  Journal of Liquid Chromatography, 24(20): 3157-3170. 2001
- Hong Wang, **Hong Jin** and Hua-shan Zhang
  Determination of catecholamines as their N-hydroxy-succinimidyl-3-indolylacetate derivatives by pre-column derivatization HPLC separation and fluorescent detection.
  Fresenius Journal of Analytical Chemistry**,** 365(8): 682-684. 1999
- **Hong Jin**, Xun Liu, Hong Wang, Hua-shan Zhang and Jie-ke Cheng
  Determination of amino acids as their N-hydroxy-succinimidyl-3-indolylacetate derivatives by pre-column derivatization HPLC separation and fluorescent detection.
  Wuhan Da Xue Xue Bao (Natural Science Journal of Wuhan University, China), 44(2): 175-178, 1998
- Guanghan Lu, **Hong Jin** and Dandan Song
  Determination of trace nitrite by anodic stripping voltammetry.
  Food Chemistry, 59(4): 583-587. 1997

List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/18WK9L8_bYGAJ/bibliography/48229889/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support:

NIH R01GM120552
Title: Mechanisms of Translational Activation by Decapping Activators
Role: Hong Jin, **P.I.**
Award period: 9/22/16 – 08/31/2021

Completed Research Support:

NIH F32GM087083 NIH Ruth L. Kirschstein National Research Service Award
Title: Characterization of Class I Release Factor-Mediated Translation Termination
Role: Hong Jin, **P.I.**
Award period: 2009-2012