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: Zhang, Jingji

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

POSITION TITLE: Post-doctoral fellow

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
UPPSALA UNIVERSITY, SWEDEN	Ph.D.	03/2016	MOLECULAR BIOLOGY
CHINA AGRICULTURE UNIVERSITY, CHINA	B.S.	07/2009	MOLECULAR BIOLOGY

A. Personal Statement

I am interested in doing ribosome translation. My primary research goal is working on protein synthesis by using classical bulky kinetic, single molecule dynamic and cryo-EM methods. The cryo-electron microscopy could study these processes of ribosome function by using authentic and functional complexes. One of my future research plans is at combining the biochemistry and structural biology; the structures can be interpreted to its corresponding physiological meanings. In each learning curve, I faced different biological knowledge, tools, and skills, but I controlled and balanced all of them every time. So I have strong learning and thinking about the ability to get new information and knowledge, and I am also skilled in an experiment using my good analytical mind to obtain more accurate and efficient results.

B. Positions and Honors

Positions

Post-doctoral Fellow 2018

Dept. of Structural Biology, Stanford University

PI: Prof. Joseph Puglisi

2016-2017

2009-2016

Post-doctoral Fellow

Dept. of Biochemistry and Molecular Biophysics, Columbia

University

PI: Prof. Joachim Frank

Ph.D. Structure and Molecular Biology

Dept. of Cell and Molecular Biology, Uppsala University

PI: Prof. Måns Ehrenberg

Honors

Wallenberg postdoc fellowship (2018 - Present)

C. Contributions to Science

- 1. Choi J, Marks J, Chen D, **Zhang J**, Wang J, Vázquez-Laslop N, Mankin A, Puglisi J. Chloramphenicol and linezolid modulate conformations of peptidyl-transfer substrates to induce context-specific translation inhibition. (In prep)
 - Studied the Chloramphenicol and linezolid induced the peptide stalling mechanisms.
- 2. Prabhakar A, **Zhang J**, Puglisi J. Coupled ribosome conformational and compositional dynamics in the mechanism of release factor 3 during translation termination. (In prep) Studied the bacterial translation termination mechanisms.
- 3. Larsen K, **Zhang J**, Puglisi J, Puglisi E. Cryo-EM studies on HIV-1 reverse transcriptase initiation complex. (In prep)
 Studied the HIV-1 reverse transcriptase initiation.
- 4. **Zhang J**, Fislage M, Mandava CS, Sanyal S, Ehrenberg M, Frank J. Cryo-EM studies on the mechanism of translation error induction by aminoglycoside antibiotics. (In prep) We determined the high-resolution structures of aminoglycoside antibiotics ribosome decoding complexes.
- 5. Fislage M, **Zhang J**, Brown ZP, Mandava CS, Sanyal S, Ehrenberg M, Frank J. Cryo-EM shows stages of initial codon selection on the ribosome by aa-tRNA in ternary complex with GTP and the GTPase-deficient EF-TuH84A. Nucleic acids research. 2018;46(11):5861-74. We determined the high-resolution structures of tRNA selection complex.
- 6. **Zhang J**, Pavlov MY, Ehrenberg M. Accuracy of genetic code translation and its orthogonal corruption by aminoglycosides and Mg2+ ions. Nucleic acids research. 2018;46(3):1362-74. Clarified the mechanism by which Mg2+ ions and antibiotic drugs of the aminoglycoside type decrease the accuracy of messenger RNA translation.
- 7. **Zhang J**, leong KW, Mellenius H, Ehrenberg M. Proofreading neutralizes potential error hotspots in genetic code translation by transfer RNAs. Rna. 2016;22(6):896-904. Shown the accuracy amplification by proofreading is essential to remedy error hot spots in initial readings of messenger RNA.
- 8. **Zhang J**, leong KW, Johansson M, Ehrenberg M. Accuracy of initial codon selection by aminoacyltRNAs on the mRNA-programmed bacterial ribosome. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(31):9602-7. There is a large variation of tRNA selection values.
- 9. Johansson M, Zhang J, Ehrenberg M. Genetic code translation displays a linear trade-off between efficiency and accuracy of tRNA selection. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(1):131-6. Used biophysical methods to demonstrate a linear trade-off between the speed and accuracy of messenger RNA translation on the bacterial ribosome.
- Wang B, Li M, Zhang J, Han C, Li D, Yu J (2008) First report of Beet soil-borne virus on sugar beet in China. Plant Pathology, 57(2):389
 Report Beet soil-borne virus in China.
- D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Wallenberg postdoc fellowship (2018 - Present)

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: Joseph D. Puglisi

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

POSITION TITLE: Professor, Dept. of Structural Biology, Stanford University

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
The Johns Hopkins University	B.A.	05/1984	Chemistry
University of California, Berkeley	Ph.D.	04/1989	Biophysical Chemistry
IBMC du CNRS, Strasbourg, France	Post Doctoral	08/1991	Biochemistry
Massachusetts Institute of Technology	Post Doctoral	07/1993	Structural Biology/ Chemistry

A. Personal Statement

I have longstanding interest in the biophysical basis of RNA function. We have developed structural and single-molecule techniques to track RNA structure and dynamics directly. Here we apply these approaches to the investigation of HIV replication.

B. Positions and Honors Positions and Employment

1993-1997	Assistant Professor, Dept. of Chemistry & Biochemistry, UC, Santa Cruz
1997	Associate Professor, Dept. of Chemistry & Biochemistry, UC, Santa Cruz
1993-1997	Affiliated Faculty, Molecular, Cellular, & Developmental Biology
	Graduate Program, Dept. of Biology, UC, Santa Cruz
1997-8/2004	Associate Professor, Department of Structural Biology
	Stanford University School of Medicine, Stanford, CA 94305-5126
1997-Present	Director, Stanford Magnetic Resonance Laboratory (SMRL)
1998-3/2004	Associate Chair, Department of Structural Biology
4-8/2004	Acting Chair, Department of Structural Biology
9/2004-2014	Chair, Department of Structural Biology
9/2004-Present	Professor of Structural Biology

Other Experience and Professional Memberships:

1984-1985	Outstanding Graduate Teaching Assistant
1989	Sigma Xi Honor Society
1994	David and Lucille Packard Fellowship in Science and Engineering
1997	Alfred P. Sloan Research Fellow

Honors

<u> </u>	
1988	Dept. of Chemistry, Berkeley, Bruce Mahan Memorial Teaching Award
1989	Thesis nominated for J.T. Baker Award in Chemistry
1993	Camille and Henry Dreyfus Teacher-Scholar Award
2014	Election, National Academy of Sciences,

C. Contribution to Science:

- Biophysical and structural Methods. Investigation of single-molecules has revolutionized our understanding of complex systems. By observing collective molecular behavior, dynamic properties obscured by ensemble averaging is revealed. We have developed several key methods to observe the dynamics of single-molecule systems. We have also a longstanding interest in applying nuclear magnetic resonance (NMR) methods to RNA structure and function.
 - a. Lukavsky PJ, **Puglisi JD** (2006). Structure Determination of Large Biological RNAs. *Methods Enzymol*; **394**:399-416.
 - b. <u>Chen J, Tsai A, Petrov A, Puglisi JD.</u> (2012). Nonfluorescent quenchers to correlate single-molecule conformational and compositional dynamics. <u>J Am Chem Soc.</u> 134(13):5734-7. PMC4369913.
 - c. Chen J, Dalal RV, Petrov AN, Tsai A, O'Leary SE, Chapin K, Cheng J, Ewan M, Hsiung PL, Lundquist P, Turner SW, Hsu DR, **Puglisi JD.** (2014). <u>High-throughput platform for real-time</u> <u>monitoring of biological processes by multicolor single-molecule fluorescence.</u> *Proc Natl Acad Sci U S A.* **111(2):**664-9. PMCID: PMC3896158.
 - d. Choi J & Puglisi JD (2017). Three tRNAs on the ribosome slow translation elongation. Proc Natl Acad Sci U S A. 2017 Dec 26;114(52):13691-13696. doi: 10.1073/pnas.1719592115. Epub 2017 Dec 11. PMID: 29229848.
- 2. Dynamics of translation. For the past 15 years, we have developed methods to explore the dynamics of protein synthesis. Our initial focus was in prokaryotic translation. We have delineated the conformational and compositional dynamics of prokaryotic translation initiation, elongation and (currently) termination. We have used these approaches to characterize the role of protein and mRNA sequence and structure in translational elongation.
 - a. Chen J, Petrov A, Johansson M, Tsai A, O'Leary SE, **Puglisi JD.** (2014). <u>Dynamic pathways of -1 translational frameshifting.</u> *Nature.* 2014 Jun 11. doi: 10.1038/nature13428. PMID: 24919156; PMC4472451.
 - b. Chen J, Coakley A, O'Connor M, Petrov A, O'Leary SE, Atkins JF, **Puglisi JD.** (2015) <u>Coupling of mRNA Structure Rearrangement to Ribosome Movement during Bypassing of Non-coding Regions. *Cell.* **163(5)**:1267-80. doi: 10.1016/j.cell.2015.10.064. PMID: 26590426.</u>
 - c. Prabhakar, A. Capece, MC, Petrov, A, Choi, J & **Puglisi JD** (2017). Post-termination ribosome intermediate acts as the gateway to ribosome recycling. Cell Rep. 2017 Jul 5;20(1):161-172. PMID: 28683310.
 - d. Choi J, Indrisiunaite G, DeMirci H, Ieong KW, Wang J, Petrov A, Prabhakar A, Rechavi G, Dominissini D, He C, Ehrenberg M, & **Puglisi JD**. (2018). <u>2'-O-methylation in mRNA disrupts tRNA decoding during translation elongation</u>. Nat Struct Mol Biol. 2018 Mar;25(3):208-216. doi: 10.1038/s41594-018-0030-z. Epub 2018 Feb 19. PMID:29459784.
- 3. **Eukaryotic Translation Initiation.** We have investigated the interplay of RNA structure and translational regulation in eukaryotic organisms. Canonical translation in eukaryotic cells occurs through recognition of the 5'cap structure through initiation factors, with subsequent scanning through the 5'-untranslated region of the RNA to the start codon. Our work has characterized the structure and function of internal ribosomal entry sites (IRES) that circumvent this process in many viruses (HCV for example). We have also investigated the dynamics of translational processes during both IRES-mediated and canonical translation initiation using single molecule approaches. The goal is a combined structural and dynamic picture for initiation occurs and is regulated.
 - a. O'Leary SE, Petrov A, Chen J, **Puglisi JD.** (2013). <u>Dynamic recognition of the mRNA cap by Saccharomyces cerevisiae elF4E.</u> *Structure.* **21(12):**2197-207. PMC3878992.
 - b. Fuchs, G., Petrov, A. N., Marceau, C. D., Popov, L. M., Chen, J., O'Leary, S. E., Wang, R., Carette, J. E., Sarnow, P., **Puglisi, JD**. (2015). Kinetic pathway of 40S ribosomal subunit recruitment to hepatitis C virus internal ribosome entry site. *Proceedings of the National Academy of Sciences of the United States of America* 112 (2): 319-325. PMC4299178.

- c. Petrov A, Grosely R, Chen J, O'Leary SE, **Puglisi JD**. (2016) <u>Multiple Parallel Pathways of Translation Initiation on the CrPV IRES.</u> Mol Cell. 2016 Apr 7;62(1):92-103. doi: 10.1016/j.molcel.2016.03.020. PMID: 27058789. PMC4826567.
- d. Johnson AG, Petrov AN, Fuchs G, Majzoub K, Grosely R, Choi J, & **Puglisi JD** (2018). Fluorescently-tagged human eIF3 for single-molecule spectroscopy. Nucleic Acids Res. 2018 Jan 25;46(2):e8. doi: 10.1093/nar/gkx1050. PMID:29136179.
- 4. Antibiotic Action. We have a longstanding interest in how small molecules can modulate RNA structure and function. Our original work 25 years ago showed how arginine could bind specifically to an HIV RNA (TAR). We then showed how aminoglycoside antibiotics bind structurally to ribosomal RNA; this work also suggested a mechanism for ribosomal decoding (aminoglycosides cause misreading of the genetic code). Recent work has used single-molecule and structural approaches to understand the dynamic perturbations of ribosomal function by antibiotics.
 - a. Fourmy, D., Recht MI, Blanchard SC, and **Puglisi JD.** (1996). Structure of the A site of *E. coli* 16S rRNA complexed with an aminoglycoside antibiotic. *Science* **274**:1367-71.
 - b. Harvey CJ, **Puglisi JD**, Pande VS, Cane DE, Khosla C. (2012). <u>Precursor directed biosynthesis of an orthogonally functional erythromycin analogue: selectivity in the ribosome macrolide binding pocket. *J Am Chem Soc.* **134(29):**12259-65. PMC3405186.</u>
 - c. Tsai A, Uemura S, Johansson M, Puglisi EV, Marshall RA, Aitken CE, Korlach J, Ehrenberg M, **Puglisi JD**. (2013). The Impact of Aminoglycosides on the Dynamics of Translation Elongation. *Cell Rep.* **3(2)**:497-508. PMC3766726.
 - d. Johansson M, Chen J, Tsai A, Kornberg G, **Puglisi JD.** (2014). <u>Sequence-dependent elongation dynamics on macrolide-bound ribosomes.</u> *Cell Rep.* **7(5):**1534-46. <u>PMC4387896.</u>
- 5. **Viral RNA structure and function.** RNA plays an outsized role in the in many viruses. We have explored RNA structure and function in HIV, HCV and other viruses. Our work used NMR spectroscopy, x-ray crystallography and cryoEM merged with single-molecule methods to understand RNA conformation and how this is modulated by viral and host proteins. We have also explored how hosts respond to viral infection through the innate immune system.
 - a. Lukavsky PJ, Kim I., Otto GA, **Puglisi JD.** (2003). Structure of HCV IRES domain II determined by NMR. *Nat Struct Biol.* **10(12):**1033-8.
 - b. McKenna SA, Lindhout DA, Kim I, Liu CW, Gelev VM, Wagner G, **Puglisi JD.** (2007). Molecular framework for the activation of RNA-dependent protein kinase. *J. Biol. Chem.*, **282(15):**11474-86. PMID: 17284445.
 - c. Puglisi EV, **Puglisi JD.** (2011). Secondary structure of the HIV reverse transcription initiation complex by NMR. *J Mol Biol.* **410(5)**:863-74. PMC3710119.
 - d. Larsen KP, Mathiharan YK, Kappel K, Coey AT, Chen DH, Barrero D, Madigan L, **Puglisi JD**, Skiniotis G, Puglisi EV. (2018). <u>Architecture of an HIV-1 reverse transcriptase initiation complex.</u> Nature. 2018 May;557(7703):118-122. doi: 10.1038/s41586-018-0055-9. Epub 2018 Apr 25. PMID:29695867.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/joseph.puglisi.1/bibliography/40645409/public/?sort=date&direction=ascending

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NAME: Prabhakar, Arjun

eRA COMMONS USER NAME (credential, e.g., agency login): arjana13

POSITION TITLE: PhD Candidate

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
UNIVERSITY OF CALIFORNIA, BERKELEY	B.S.	05/2013	CHEMICAL BIOLOGY

A. Personal Statement

Coming from a chemistry background in college, I have always been fascinated by the efficiency with which biomolecules enzymatically synthesize molecules of all shapes and sizes. One beautiful illustration of this phenomenon is how large macromolecular assemblies like ribosomes can synthesize polypeptides with high efficiency and accuracy. Coming to Stanford for my PhD in Biophysics, I had the opportunity to study this fundamental process of translation in Joseph Puglisi's lab using the cutting-edge technology of real-time single-molecule fluorescence. The last five years of training in this technique in the Puglisi lab has given me the ability to construct new fluorescence-based assays by functionalizing protein and RNA with fluorescent probes. My focus on uncovering the dynamics of translation has also given me a new perspective about the crucial role timing and rates play in multi-step biological processes like translation. I have also come to appreciate the power of biophysical tools like single-molecule fluorescence and cryo-electron microscopy in illustrating how the architecture of macromolecular assemblies relates to the multifaceted nature of their mechanisms. With my newfound perspective in biophysics and structural biology, I seek to continue my interests in developing new biophysical tools to study complex biological processes.

B. Positions and Honors

Positions

Graduate Research Assistant, Stanford University (2013-Present)

Honors

Affymetrix Bio-X Stanford Interdisciplinary Graduate Fellowship (2016 – Present) NSF Graduate Research Fellowship Program Honorable Mention (2013, 2014, 2015)

C. Contributions to Science

- 1. Studying the mechanism of bacterial translation termination and ribosome recycling
 My primary thesis project has been to uncover the dynamics at play during the late stages of bacterial
 translation. Studying the late stages of termination and ribosome recycling using single-molecule
 fluorescence required engineering of fluorescently-tagged constructs of the protein factors involved in
 these processes. With these new tools, I have expanded the existing single-molecule assay in the lab
 to now be able to monitor all four stages of translation in bacteria (initiation, elongation, termination, and
 recycling) in real time.
 - e. Prabhakar A, Capece MC, Petrov A, Choi J, Puglisi JD (2017) Post-termination ribosome intermediate acts as the gateway to ribosome recycling. Cell Rep. 2017 Jul 5;20(1):161-172. PMID: 28683310.
- 2. Reagent development and data analysis for other single-molecule fluorescence projects.
 I have contributed in other projects where I had assisted in the development of reagents and analysis of data collected from single-molecule fluorescence experiments.
 - a. Choi J, Ieong KW, DeMirci H, Chen J, Petrov A, Prabhakar A, O'Leary SE, Dominissini D, Rechavi G, Soltis SM, Ehrenberg M, & Puglisi JD. (2016). N(6)-methyladenosine in mRNA disrupts tRNA selection and translation-elongation dynamics. Nat Struct Mol Biol. 2016 Feb;23(2):110-5. PMID: 26751643.
 - b. Choi J, Indrisiunaite G, DeMirci H, Ieong KW, Wang J, Petrov A, Prabhakar A, Rechavi G, Dominissini D, He C, Ehrenberg M, & Puglisi JD. (2018). 2'-O-methylation in mRNA disrupts tRNA decoding during translation elongation. Nat Struct Mol Biol. 2018 Mar;25(3):208-216. PMID:29459784.
 - c. Wang J, Johnson AG, Lapointe CP, Choi J, Prabhakar A, Chen DH, Petrov AN, Puglisi JD (2019) eIF5B gates the transition from translation initiation to elongation. (Submitted)

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing

Affymetrix Bio-X Stanford Interdisciplinary Graduate Fellowship (2016 – Present)

Previous

NIH Molecular Biophysics Pre-doctoral Research Training Grant, T32-GM008294 (2013 – 2016)

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NAME: Fu, ZIAO

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

POSITION TITLE: GRADUATE STUDENT

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
JILIN UNIVERSITY, CHANGCHUN, CHINA	B.S.	2012	CHEMISTRY

A. Personal Statement

I have been working in the cryo-EM field for five years in Joachim Frank's lab as a graduate student. Mainly I am working on time-resolved cryo-EM method development and application. I showed a few successful cases to demonstrate the time-resolved cryo-EM method can capture short-lived intermediates which have half-life time from 10 ms to 1 s. Apart from this main project I am working on, I am also involved in a few other projects to help the researchers to succeed in solving structures using cryo-EM technique. One exciting example is a collaboration with Youzhong Guo and Wayne Hendrickson's lab on membrane protein extraction method development. Avoiding detergent in protein extraction from cell membrane, we can preserve native lipids and we observed for the native lipid bilayer directly extracted from native cell membrane. The method would help researchers working with membrane protein to gain more insight in terms of protein lipid interaction and lipid functional and structure roles.

B. Positions and Honors

Research Assistant 2014-2018 Columbia University.

C. Contributions to Science

 The structural basis for release factor activation during translation termination revealed by time resolved cryo-EM. 2018 <u>Ziao Fu*</u>, Gabriele Indrisiunaite*, Sandip Kaledhonkar*, Binita Shah, Ming Sun, Bo Chen, Robert A. Grassucci, Måns Ehrenberg, Joachim Frank (in review)

We determined high-resolution structures of short-lived intermediates in the translation termination process using time-resolved Cryo-EM technique.

2. Real-time structural dynamics of late steps in bacterial translation initiation visualized using time-resolved cryogenic electron microscopy. 2018 Sandip Kaledhonkar*, Ziao Fu*, Kelvin Caban*, Wen Li, Bo Chen, Ming Sun, Ruben Gonzalez Jr, Joachim Frank (Nature in press) We determined high-resolution structures of short-lived intermediates in the translation initiation process using time-resolved Cryo-EM technique.

- 3. Structure and Activity of Lipid Bilayer within a Membrane Protein Transporter 2018 Weihua Qiu*, Ziao Fu*, Guoyan G. Xu, Robert A. Grassucci, Yan Zhang, Joachim Frank, Wayne A. Hendrickson, Youzhong Guo Proc Natl Acad Sci U S A. 2018 Dec 18;115(51):12985-12990.
 We solved native lipid bilayer structure by Cryo-EM technique at high resolution about 3 A.
- 4. A Fast and Effective Microfluidic Spraying-Plunging Method for High-Resolution Single-Particle Cryo-EM 2017 Xiangsong Feng*, Ziao Fu*, Sandip Kaledhonkar, Yuan Jia, Binita Shah, Amy Jin, Zheng Liu, Ming Sun, Bo Chen, Robert A Grassucci, Yukun Ren, Hongyuan Jiang, Joachim Frank, Qiao Lin Structure 25 (4), 663-670. e3
 We describe a spraying-plunging method for preparing cryoelectron microscopy (cryo-EM) grids with vitreous ice of controllable, highly consistent thickness using a microfluidic device. The new polydimethylsiloxane (PDMS)-based sprayer was tested with apoferritin. We demonstrate that the structure can be solved to high resolution with this method of sample preparation. Besides replacing the conventional pipetting-blotting-plunging method, one of many potential applications of the new sprayer is in time-resolved cryo-EM, as part of a PDMS-based microfluidic reaction channel to study short-lived intermediates on the timescale of 10-1,000 ms.
- 5. Key intermediates in ribosome recycling visualized by time-resolved cryoelectron microscopy 2016 Ziao Fu*, Sandip Kaledhonkar*, Anneli Borg*, Ming Sun, Bo Chen, Robert A Grassucci, Måns Ehrenberg, Joachim Frank Structure 24 (12), 2092-2101

We determined the structures of short-lived intermediates in the translation recycling process using time-resolved cryo-EM technique. Upon encountering a stop codon on mRNA, polypeptide synthesis on the ribosome is terminated by release factors, and the ribosome complex, still bound with mRNA and P-site-bound tRNA (post-termination complex, PostTC), is split into ribosomal subunits, ready for a new round of translational initiation. Separation of post-termination ribosomes into subunits, or "ribosome recycling," is promoted by the joint action of ribosome-recycling factor (RRF) and elongation factor G (EF-G) in a guanosine triphosphate (GTP) hydrolysis-dependent manner. Here we used a mixing-spraying-based method of time-resolved cryo-electron microscopy (cryo-EM) to visualize the short-lived intermediates of the recycling process. The two complexes that contain (1) both RRF and EF-G bound to the PostTC or (2) deacylated tRNA bound to the 30S subunit are of particular interest. Our observations of the native form of these complexes demonstrate the strong potential of time-resolved cryo-EM for visualizing previously unobservable transient structures.

D. Additional Information: Research Support and/or Scholastic Performance

Not applicable.

I am supported by this grant. But I don't know if I should list it here or how to describe my role in the grant.

Ongoing

RO1 GM029169 JOACHIM FRANK, PI 1994 – 2019

NIH NIGMS

STRUCTURAL ANALYSIS OF MACROMOLECULAR ASSEMBLIES

This study explores structure and function of the ribosome actively engaged in protein synthesis, by cryo-electron microscopy (cryo-EM) and single-particle reconstruction.