BIOGRAPHICAL SKETCH

NAME: ATOUSA MEHRANI

POSITION TITLE: PHD CANDIDATE

EDUCATION/TRAINING:

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	START DATE MM/YYYY	COMPLETION DATE MM/YYYY	FIELD OF STUDY
Tehran Azad University, Science and Research Branch (SRB), Iran	B.Sc.	09/2008	09/2008	Engineering Physics
Queen Mary, University of London, United Kingdom	M.Sc.	09/2012	09/2014	Biomedical Engineering
Florida State University, United States	Ph.D. Candidate	08/2016	Present	Biochemistry

A. Personal Statement

I have a long-standing and strong interest in the structural analysis of biological complexes by cryo-electron microscopy (cryoEM). My current Ph.D. research is focused on the structural analysis of the stalled bacterial ribosome bound to a drug molecule that inhibits rescue mechanism in ribosomes, using cryoEM as well as the structural analysis of *in situ* vesicular transport protein by cryo-electron tomography (cryoET). In the course of my Ph.D. program in the lab of Scott Stagg at Florida State University, I had the opportunity to develop my skills in the field of cryoEM. I have gained expertise in sample preparation and single particle reconstruction techniques. I also developed some experience in tomographic reconstruction. My goal is to improve my knowledge and experience in both areas of single particle and tomographic reconstruction to implement these methods in drug design and in various aspects of biomedical sciences, which may ultimately to be used for human well-being.

B. Positions and Honors

Positions and Employment

2017-Present	Graduate Research Assistant	Florida State University, USA
2015-2016	Graduate Researcher	Tehran Azad University, SRB, Iran
2014-2015	Graduate Researcher	Tehran Azad University, SRB, Iran
2013-2014	Graduate Researcher	Queen Mary, University of London, UK
2011-2011	Undergraduate Researcher	Tehran Azad University, SRB, Iran

Professional Memberships

2017-Present Member, Biophysical Society
2018-2017 Member, Congress of Graduate Students, Florida State University

C. Contribution to Science

1. Structural mechanism of the stalled bacterial ribosome bound to a trans-translation inhibitor using cryoEM

The aim of this project is to determine the structure of the E. Coli ribosome together with a drug molecule that inhibits trans-translation. Trans-translation is one of the fundamental rescue mechanisms used by the stalled bacterial ribosome and it is important in many pathogenic strains. Therefore, trans-translation has been proposed as a target for the development of new antibiotics. Small molecules have been synthesized that possess antibiotic properties and inhibit rescue mechanism in the stalled ribosomes; however, the action mechanism used by these drug molecules is unknown. Rational improvement of these drug compounds and furthermore the design of new antibiotics that target ribosome rescue or trans-translation requires detailed structural information of the target binding sites. By using cryoEM data analysis and single particle reconstruction, I have resolved the binding site of these drug molecules within E. Coli 70S ribosome. I have also observed a novel conformation of the ribosomal protein (L27), which may explain the function of the drug as well as the catalysis mechanism used by stalled ribosomes. The results of this research have been presented as a poster presentation at the 2019 Biophysical Society meeting and the manuscript for this research work is currently in preparation for submission to a peer-reviewed journal. This project is run in collaboration with Dunham Lab at Emory University and Keiler Lab at Pennsylvania State University.

a. Mehrani, A., Hoffer, E.D., Goralski, T.D.P., Keiler, K.C., Dunham, Ch., Stagg, S. Investigating the structural mechanism of the stalled bacterial ribosome bound to a drug that targets trans-translation. Biophysical Journal 116(3):573a-574a · February 2019. DOI: 10.1016/j.bpj.2018.11.3085

b. Mehrani, A., Hoffer, E.D., Goralski, T.D.P., Keiler, K.C., Dunham, Ch., Stagg, S. CryoEM structural analysis of the stalled *E-coli* ribosome bound to trans-translation inhibitor (KKL2908). (Manuscript in preparation).

2. Structural analysis of *in situ* vesicular transport proteins by cryoET

This project is engaged with investigating the *in situ* organization of the vesicular transport proteins including tyrosine-receptor kinase fused gene (TFG) within in the secretory pathway by using cryo-electron tomography. TFG is known as a conserved regulator of protein secretion, while its mutation is associated with oncogenesis and neurodegenerative disorders. Since this protein is concentrated near the nucleus site of the eukaryotic cells, using cryo-focused ion beam milling (cryo-FIB-milling) is essential to produce thin slices of the cells that are detectable by mean of cryoEM. In collaboration with Alex Noble at NYSBC, we made several FIB-milled lamellae of a eukaryotic cell and collected several tilt-series, which were then subjected to the

reconstruction of 3D tomograms. In the course of this project, I had an opportunity to spend a couple of days at NYSBC to gain insight into the FIB-milling procedure. Our reconstructed tomograms had sufficient resolution to illustrate several organelles in the secretory pathway of the cell. However, considering the small size of the TFG proteins, which are buried in the dense sea of the surrounding molecules, we required much higher resolution to capture the TFG protein *in situ* and reconstruct it by sub-tomogram averaging. Since then several measurements have been taken to improve the sample preparation and our imaging techniques to facilitate the observation of TFG during the cryoEM data collection. This project is run in collaboration with Audhya Lab at Wisconsin-Madison and Alex Noble at NYSBC.

3. Biomaterial synthesis and characterization for medical imaging and drug delivery

During my master's degree and post graduation, I had been involved in several research projects associated with the fabrication of micro/nanocarriers with applications in the drug delivery and medical imaging. In the lab of Gleb Sukhorokov at Queen Mary, University of London I developed skills in fabricating optical responsive polymeric microcapsules by layer-by-layer assembly technique. These microcapsules had hollow structures and could be loaded with various enzymes and drugs. If the capsules were embedded by magnetic nanoparticles, they could be guided to the specific site *in vitro* and *in vivo* by using a magnetic field to deliver their cargoes to the site, such as cancerous cells. Following this project, I got two internship positions in Iran that were involved with the fabrication of metal nanocomposites using laser ablation. Due to the optical properties, these nanocomposites are potential to be used as contrast agents to improve the contrast and quality of the MRI imaging. Flowing that, I was involved in a research project associated with the development of the magnetic nanocarriers. These nanoparticles had a porous structure and their surface property could be modified by various functional groups. These nanocarriers were loaded with doxorubicin (an anti-cancer drug) and their drug release mechanism was studied *in vitro*. The last two research projects were published in peer-reviewed journals.

a. Mehrani, A., Dorranian, D. & Solati, E. J Clust Sci (2015) 26: 1743. Properties of Au/ZnO Nanocomposite Prepared by Laser Irradiation of the Mixture of Individual Colloids DOI: 10.1007/s10876-015-0872-z.

b. Mehrani, A., Ahmadvand, P., Mehdizadeh Barforushi, M. et al. J Inorg Organomet Polym (2016) 26: 226. Double Functionalized Nanoporous Magnetic Gadolinium–Silica Composite for Doxorubicin Delivery. DOI:10.1007/s10904-015-0302-6.

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

Recent Graduate Courses

YEAR	COURSE TITLE	GRADE
2016	Programing for Chemists and Biochemists (Modules in Python)	A+
2016	Quantum Mechanics	B+
2017	Selected Topics: Genetics and Cell Biology (Modules in CryoEM)	A+
2017	Molecular Biology	B+

2017	Structure and Function of Enzymes	B
2018	Scientific Writing and Presentation	S

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: STAGG, SCOTT M

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

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)	END DATE MM/YYYY	FIELD OF STUDY
Oglethorpe University	BS	06/1996	Biology
University of Alabama at Birmingham	PHD	12/2002	Biochemistry
Georgia Institute of Technology	Postdoctoral Fellow	2003	Biophysics
The Scripps Research Institute	NIH training grant	2007	Structural biology

A. Personal Statement

I have many years experience in 3DEM, and my lab is very well suited to carry out high-resolution 3DEM structure determination. I am a developer of the Leginon and Appion software packages for automated cryo-EM and my lab has used that software to determine the structures of COPII complexes and to systematize the data acquisition and processing to drive the EM reconstruction process to higher resolution. At FSU, we are equipped with an FEI Titan Krios electron microscope that is outfitted with a DE64 counting electron detector, a K3 energy filtered counting detector, and the Leginon software package for automated high-throughput data collection. Our highest resolution cryo-EM reconstruction to date is 2.7 Å. My lab is currently interested in developing new tools to enable high-throughput high-resolution cryo-EM, and to use those tools to further our understanding of the functional mechanisms that facilitate vesicle formation in the cell.

1. Paraan M, Bhattacharya N, Uversky VN, Stagg SM. Flexibility of the Sec13/31 cage is influenced by the Sec31 C-terminal disordered domain. J Struct Biol. 2018 Nov;204(2):250-260. PubMed PMID: 30172710; PubMed Central PMCID: PMC6188663.
2. Mendez JH, Stagg SM. Assessing the quality of single particle reconstructions by atomic model building. J Struct Biol. 2018 Nov;204(2):276-282. PubMed PMID: 30213768; PubMed Central PMCID: PMC6201253.
3. Xie Q, Spear JM, Noble AJ, Sousa DR, Meyer NL, Davulcu O, Zhang F, Linhardt RJ, Stagg SM, Chapman MS. The 2.8 Å Electron Microscopy Structure of Adeno-Associated Virus-DJ Bound by a Heparinoid Pentasaccharide. Mol Ther Methods Clin Dev. 2017 Jun 16;5:1-12. PubMed PMID: 28480299; PubMed Central PMCID: PMC5415311.
4. Stagg SM, Frazer-Abel AA, Hagerman PJ, Harvey SC. Structural studies of the tRNA domain of tmRNA. J Mol Biol. 2001 Jun 8;309(3):727-35. PubMed PMID: 11397092.

B. Positions and Honors

Positions and Employment

2004 - 2007 Postdoctoral Fellow, The Scripps Research Institute
2007 - 2014 Assistant Professor, Florida State University
2014 - Associate Professor, Florida State University

Other Experience and Professional Memberships

2002 - Member, Biophysical Society
2008 - Member, American Society for Cell Biology
2009 - 2010 Member, Study Section for Technology Development, DOE

2011 - 2012	Member, Ad Hoc Panel for PPG, NIH
2013 - 2014	Ad Hoc Reviewer FEC Fellowship, MRC
2013 - 2014	Ad Hoc Reviewer CDA fellowship, HFSP
2014 -	Member, Microscopy Society of America
2014 - 2015	Ad Hoc Reviewer, BDMA study section, NIH
2015 - 2016	Ad Hoc Reviewer, AHA Postdoctoral Fellowship, AHA
2015 - 2016	Ad Hoc Reviewer, SYN study section, NIH
2016 - 2016	Ad Hoc Reviewer, MSFC study section, NIH
2016 - 2016	Ad Hoc Reviewer, MSFB study section, NIH
2016 - 2016	Reviewer, BCMB PPG study section, NIH
2017 - 2017	Ad Hoc Reviewer, BBSRC
2017 - 2017	Reviewer, NC Biotech Center
2017 - 2017	Reviewer, ZRG1 IMST-L 50 study section, NIH
2018 - 2018	Reviewer, SIG study section, NIH
2018 - 2018	Reviewer, SBIR review panel, DOE

Honors

1992	Oglethorpe Scholars, Oglethorpe University
2008	First Year Assistant Professor Award, Florida State University
2016	Developing Scholar Award, Florida State University

C. Contribution to Science

1. My early research centered on the structure of the ribosome and the interactions with its cofactors. Highlights of these studies include modeling the tRNA domain of tmRNA, interpreting the 30S ribosomal subunit assembly map in terms of its structure, and simulating the interdomain flexibility of ribosome recycling factor. Part of this work involved a collaboration with the lab of Dr. Joachim Frank on interpreting his cryoEM maps of the ribosome in terms of atomic structure. This is one of the earliest examples of the power of modeling for interpreting cryoEM maps. Dr. Frank's group produced cryoEM maps of the ribosome in two different states, and these showed a dramatic conformational change in the tRNA. I modeled the atomic coordinates of the tRNA in both conformational states, and this helped us to understand the structural mechanism by which the ribosome selects and accommodates incoming tRNA during translation.

    a. Stagg SM, Frazer-Abel AA, Hagerman PJ, Harvey SC. Structural studies of the tRNA domain of tmRNA. J Mol Biol. 2001 Jun 8;309(3):727-35. PubMed PMID: 11397092.
    b. Stagg SM, Mears JA, Harvey SC. A structural model for the assembly of the 30S subunit of the ribosome. J Mol Biol. 2003 Apr 18;328(1):49-61. PubMed PMID: 12683996.
    c. Gao H, Sengupta J, Valle M, Korostelev A, Eswar N, Stagg SM, Van Roey P, Agrawal RK, Harvey SC, Sali A, Chapman MS, Frank J. Study of the structural dynamics of the E coli 70S ribosome using real-space refinement. Cell. 2003 Jun 13;113(6):789-801. PubMed PMID: 12809609.
    d. Valle M, Zavialov A, Li W, Stagg SM, Sengupta J, Nielsen RC, Nissen P, Harvey SC, Ehrenberg M, Frank J. Incorporation of aminoacyl-tRNA into the ribosome as seen by cryo-electron microscopy. Nat Struct Biol. 2003 Nov;10(11):899-906. PubMed PMID: 14566331.

2. One of the aims in my research career has been to create tools for facilitating high-throughput high-resolution 3D electron microscopy (3DEM). Going from sample to 3D reconstruction can be an incredibly tedious and time consuming process in 3DEM. In pursuit of automating the process of data collection and processing, I have had a role in the development of the Leginon software package that is distributed by the National Resource for Automated Microscopy (NRAMM). Leginon is one of the most highly used software packages for automated cryo-EM data collection. Furthermore, when I was working at the NRAMM, I together with Gabriel Lander and Neil Voss created a software package called Appion that provides tools for automated data processing and integrates some of the many other image processing packages so that

data can be processed in different packages and the results compared in a seamless high-throughput and automated manner.

a. Mendez JH, Stagg SM. Assessing the quality of single particle reconstructions by atomic model building. J Struct Biol. 2018 Nov;204(2):276-282. PubMed PMID: 30213768; PubMed Central PMCID: PMC6201253.
b. Shrum DC, Woodruff BW, Stagg SM. Creating an infrastructure for high-throughput high-resolution cryogenic electron microscopy. J Struct Biol. 2012 Oct;180(1):254-8. PubMed PMID: 22842049; PubMed Central PMCID: PMC3466351.
c. Lander GC, Stagg SM, Voss NR, Cheng A, Fellmann D, Pulokas J, Yoshioka C, Irving C, Mulder A, Lau PW, Lyumkis D, Potter CS, Carragher B. Appion: an integrated, database-driven pipeline to facilitate EM image processing. J Struct Biol. 2009 Apr;166(1):95-102. PubMed PMID: 19263523; PubMed Central PMCID: PMC2775544.
d. Suloway C, Pulokas J, Fellmann D, Cheng A, Guerra F, Quispe J, Stagg S, Potter CS, Carragher B. Automated molecular microscopy: the new Leginon system. J Struct Biol. 2005 Jul;151(1):41-60. PubMed PMID: 15890530.

3. A major focus of my career as an independent investigator has been determining the structures and mechanisms of the COPII proteins that are involved in transport of secreted cargo between the ER and the Golgi apparatus. The COPII coat is comprised of five cytosolic proteins, Sar1, Sec23, Sec24, Sec13, and Sec31 that together form a coat on the ER and gather cargo proteins into a vesicle that is transported to the Golgi apparatus. We have determined structures of the Sec13/31 COPII cage, the Sec23/24-Sec13/31 COPII coat, a tubular Sec13/31 structure with potential for carrying elongated cargo, and the Sar1 lattice that is implicated in vesicle fission. We combine our structural studies with biochemistry to elucidate the mechanisms by which the COPII complex functions.

a. Paraan M, Bhattacharya N, Uversky VN, Stagg SM. Flexibility of the Sec13/31 cage is influenced by the Sec31 C-terminal disordered domain. J Struct Biol. 2018 Nov;204(2):250-260. PubMed PMID: 30172710; PubMed Central PMCID: PMC6188663.
b. Johnson A, Bhattacharya N, Hanna M, Pennington JG, Schuh AL, Wang L, Otegui MS, Stagg SM, Audhya A. TFG clusters COPII-coated transport carriers and promotes early secretory pathway organization. EMBO J. 2015 Mar 12;34(6):811-27. PubMed PMID: 25586378; PubMed Central PMCID: PMC4369316.
c. Noble AJ, Zhang Q, O'Donnell J, Hariri H, Bhattacharya N, Marshall AG, Stagg SM. A pseudoatomic model of the COPII cage obtained from cryo-electron microscopy and mass spectrometry. Nat Struct Mol Biol. 2013 Feb;20(2):167-73. PubMed PMID: 23262493; PubMed Central PMCID: PMC3565055.
d. Stagg SM, Gürkan C, Fowler DM, LaPointe P, Foss TR, Potter CS, Carragher B, Balch WE. Structure of the Sec13/31 COPII coat cage. Nature. 2006 Jan 12;439(7073):234-8. PubMed PMID: 16407955.

4. I have had a long-time interest in determining what are the factors that limit resolution in single particle reconstructions. We have used GroEL and adeno-associated virus (AAV) as test systems to probe the data collection and processing parameters for cryo-EM and empirically determine optimal methods for collecting and processing data in a systematic way. Recently, we have developed metrics based on what we call ResLog plots that report on the data quality and reconstruction quality for 3D reconstructions. These metrics can be used to validate reconstructions and drive to high resolution for challenging macromolecules.

a. Stagg SM, Mendez JH. Processing apoferritin with the Appion pipeline. J Struct Biol. 2018 Oct;204(1):85-89. PubMed PMID: 29969662; PubMed Central PMCID: PMC6119502.
b. Stagg SM, Noble AJ, Spilman M, Chapman MS. ResLog plots as an empirical metric of the quality of cryo-EM reconstructions. J Struct Biol. 2014 Mar;185(3):418-26. PubMed PMID: 24384117; PubMed Central PMCID: PMC4001718.
c. Lerch TF, O'Donnell JK, Meyer NL, Xie Q, Taylor KA, Stagg SM, Chapman MS. Structure of AAV-DJ, a retargeted gene therapy vector: cryo-electron microscopy at 4.5 Å resolution. Structure. 2012 Aug 8;20(8):1310-20. PubMed PMID: 22727812; PubMed Central PMCID: PMC3418430.

d. Stagg SM, Lander GC, Quispe J, Voss NR, Cheng A, Bradlow H, Bradlow S, Carragher B, Potter CS. A test-bed for optimizing high-resolution single particle reconstructions. J Struct Biol. 2008 Jul;163(1):29-39. PubMed PMID: 18534866; PubMed Central PMCID: PMC2505049.

5. Recently, my lab has been involved in determining the structures and mechanisms utilized by CRISPR complexes. CRISPR complexes comprise a large family of bacterial and archaeal proteins that use their nuclease activity to target the nucleic acid of infecting phages. In collaboration with the labs of Hong Li and Becky and Michael Terns, we have determined the structures of various Cmr complex structures and have determined the structural and mechanistic roles of many of the 6 proteins that comprise the complex.

   a. Spilman M, Cocozaki A, Hale C, Shao Y, Ramia N, Terns R, Terns M, 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.
   b. Ramia NF, Spilman M, Tang L, Shao Y, Elmore J, Hale C, Cocozaki A, Bhattacharya N, Terns RM, Terns MP, Li H, Stagg SM. Essential structural and functional roles of the Cmr4 subunit in RNA cleavage by the Cmr CRISPR-Cas complex. Cell Rep. 2014 Dec 11;9(5):1610-1617. PubMed PMID: 25482566; PubMed Central PMCID: PMC4269474.

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

Ongoing Research Support

Subaward from GM110567, NIH
STAGG, SCOTT M (PI)
03/01/15-02/28/20
Regulatory mechanisms that control vesicle secretion at the endoplasmic reticulum
The overall goals of this grant are to determine the role of the protein complex TFG in organizing ER exit sites in the secretory pathway. Our role in the project is to determine the structures of TFG complexes purified by the lab of Anjon Audya (PI on the grant).
Role: Co-Investigator

U24 GM116788, NIH
STAGG, SCOTT M (PI)
07/18/16-06/30/20
The Southeastern Consortium for Microscopy of MacroMolecular Machines
Role: CPI

R01 GM108753
STAGG, SCOTT M (PI)
09/30/15-09/29/16
Tools for High-Throughput High-Resolution Three Dimensional Electron Microscopy
Role: PI