BIOGRAPHICAL SKETCH

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NAME: STAGG, SCOTT M

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

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)	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 over a decade of experience in 3DEM, and my expertise is very well suited to implement the new proposed instrumentation. 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. My lab had one of the first sub 3Å 3D reconstructions at the advent of the cryo-EM in 2015. At FSU, we are equipped with an FEI Titan Krios electron microscope that is outfitted with two counting direct electron detectors, the DE64 and energy filtered K3, and the Leginon software package for automated high-throughput data collection. The combination of the high- resolution and stability of the Krios with Leginon/Appion creates a high-throughput high-resolution pipeline that is the backbone on which all of the EM labs at FSU collect and process their data. It is also the backbone that drives data collection and processing for the current Southeastern Consortium for Microscopy of Macromolecular Machines (SECM4) that is hosted at FSU. The technical expertise from my lab that has contributed to this research will be invaluable for setting up and expanding the new SECM4. On the biological side, my lab is interested in determining the structures and mechanisms of complexes in the secretory pathway. We have been studying the structures of COPII complexes assembled in vitro, the structures of tubular complexes that are involved in membrane remodeling, and the structures of natively assembled clathrin coated vesicles.

1. Paraan M, Mendez J, Sharum S, Kurtin D, He H, Stagg SM. The Structures of Natively Assembled Clathrin Coated Vesicles. Science Advances. Forthcoming.
2. Randolph PS, Stagg SM. Reconstruction of Average Subtracted Tubular Regions (RASTR) Enables Structure Determination of Tubular Filaments by Cryo-EM. JSBx. Forthcoming.
3. Mendez JH, Mehrani A, Randolph P, Stagg S. Throughput and resolution with a next-generation direct electron detector. IUCrJ. 2019 Nov 1;6(Pt 6):1007-1013. PubMed Central PMCID: PMC6830211.
4. 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 Central PMCID: PMC6201253.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2020 - Professor, Florida State University

2014 - 2020 Associate Professor, Florida State University
2007 - 2014 Assistant Professor, Florida State University
2004 - 2007 Postdoctoral Fellow, The Scripps Research Institute

Honors

2016 Developing Scholar Award, Florida State University
2008 First Year Assistant Professor Award, Florida State University
1992 Oglethorpe Scholars, Oglethorpe 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. Recently, we initiated a collaboration with the labs of Drs Christine Dunham and Graeme Conn to determine the structures of ribosomes by cryo-EM.

    a. Nosrati M, Dey D, Mehrani A, Strassler SE, Zelinskaya N, Hoffer ED, Stagg SM, Dunham CM, Conn GL. Functionally critical residues in the aminoglycoside resistance-associated methyltransferase RmtC play distinct roles in 30S substrate recognition. J Biol Chem. 2019 Nov 15;294(46):17642-17653. PubMed Central PMCID: PMC6873201.
    b. 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.
    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. 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.

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. My lab has used the Leginon/Appion environment to create new tools to enable cryo-EM structure determination of challenging specimens, facilitate better-faster reconstructions, and to incorporate and characterize new cameras for use by the cryo-EM field.

    a. Randolph P, Stagg S. Reconstruction of Average Subtracted Tubular Regions (RASTR) enables structure determination of tubular filaments by cryo-EM. Journal of Structural Biology: X. 2020;

4:100023-. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2590152420300052 DOI: 10.1016/j.yjsbx.2020.100023

b. Mendez JH, Mehrani A, Randolph P, Stagg S. Throughput and resolution with a next-generation direct electron detector. IUCrJ. 2019 Nov 1;6(Pt 6):1007-1013. PubMed Central PMCID: PMC6830211.

c. Noble AJ, Stagg SM. Automated batch fiducial-less tilt-series alignment in Appion using Protomo. J Struct Biol. 2015 Nov;192(2):270-8. PubMed Central PMCID: PMC4633401.

d. 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 Central PMCID: PMC4001718.

3. A major focus of my career as an independent investigator has been determining the structures and mechanisms of vesicle trafficking complexes. We have focused primarily on 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. Recently we have shifted focus to clathrin coated vesicles and have determined the structures of natively assembled coated vesicles which revealed structure of a novel clathrin geometry and showed how adaptor appendages crosslink adjacent clathrin triskelia to promote assembly of clathrin cages.

   a. Paraan M, Mendez J, Sharum S, Kurtin D, He H, Stagg SM. The Structures of Natively Assembled Clathrin Coated Vesicles. Science Advances. Forthcoming.

   b. 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 Central PMCID: PMC6188663.

   c. Hariri H, Bhattacharya N, Johnson K, Noble AJ, Stagg SM. Insights into the mechanisms of membrane curvature and vesicle scission by the small GTPase Sar1 in the early secretory pathway. J Mol Biol. 2014 Nov 11;426(22):3811-3826. PubMed Central PMCID: PMC4254083.

   d. 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 Central PMCID: PMC3565055.

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 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 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 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 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 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 Central PMCID: PMC4269474.

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