BIOGRAPHICAL SKETCH

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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	12/2003	Biophysics
The Scripps Research Institute	NIH training grant	08/2007	Structural biology

A. Personal Statement

My role in the proposed research is to characterize the structures of the TFG complexes using three-dimensional electron microscopy (3DEM) techniques. I have many years experience in 3DEM, and my lab is very well suited to carry out the proposed studies. My lab specializes in determining the structures of COPII coat components and in high-throughput high-resolution cryo-EM. At FSU, we have a state-of-the-art Titan Krios electron microscope equipped with a DE64 direct detector with counting mode and a Gatan K3 BioQuantum, and also equipped with a Volta phase plate. This system will allow us to optimize the conditions for imaging the TFG complexes so that we can determine the highest resolution structure possible. Moreover, studies of TFG mesh well with the main research themes of my lab. My lab is involved in determining the structure and mechanisms of COPII coated vesicles that are players in the early secretory pathway. One of the big questions in the early secretory pathway is how does Sec16 act to spatially regulate the formation of COPII vesicles. Dr. Audhya has determined that TFG interacts with Sec16, and participates in spatially organizing the formation of COPII coated vesicles in the cell. Determining the structure of TFG and characterizing its interaction with Sec16 will synergize well with our COPII research to advance the field of vesicle trafficking.

1. Lowry TW, Hariri H, Prommapan P, Kusi-Appiah A, Vafai N, Bienkiewicz EA, Van Winkle DH, Stagg SM, Lenhert S. Quantification of Protein-Induced Membrane Remodeling Kinetics In Vitro with Lipid Multilayer Gratings. Small. 2016 Jan 27;12(4):506-15. PubMed PMID: 26649649; PubMed Central PMCID: PMC4843995.
2. 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 PMID: 25193674; PubMed Central PMCID: PMC4254083.
3. 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.
4. 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.

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, ZRG1 study section, NIH

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. Spear JM, Noble AJ, Xie Q, Sousa DR, Chapman MS, Stagg SM. The influence of frame alignment with dose compensation on the quality of single particle reconstructions. J Struct Biol. 2015 Nov;192(2):196-203. PubMed PMID: 26391007; PubMed Central PMCID: PMC4633374.
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. 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.
b. 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.
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 PMID: 25193674; PubMed Central PMCID: PMC4254083.
d. 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.

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, 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.
b. 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.

c. Xie Q, Spilman M, Meyer NL, Lerch TF, Stagg SM, Chapman MS. Electron microscopy analysis of a disaccharide analog complex reveals receptor interactions of adeno-associated virus. J Struct Biol. 2013 Nov;184(2):129-35. PubMed PMID: 24036405; PubMed Central PMCID: PMC3866096.

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 PMID: 24384117; PubMed Central PMCID: PMC4001718.

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.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/scott.stagg.1/bibliography/40832943/public/

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

Ongoing Research Support

R01 GM108753, NIH STAGG, SCOTT M (PI) 09/30/15 - 06/30/21
Tools for High-Throughput High-Resolution Three Dimensional Electron Microscopy
Role: PI

U24 GM116788, NIH TAYLOR, KENNETH (PI) 07/18/16-06/30/20
The Southeastern Consortium for Microscopy of MacroMolecular Machines
Role: Co-I

Subaward from GM110567, NIH AUDHYA, ANJON (PI) 03/01/15-02/28/20
Regulatory mechanisms that control vesicle secretion at the endoplasmic reticulum
Role: PI on subaward

Subaward from GM093278, NIH DUNHAM, CHRISTINE (PI) 02/01/2017- 05/31/2019
Structural Studies of Ribosome Regulation
Role: PI on subaward

BIOGRAPHICAL SKETCH

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NAME: **Anjon Audhya**

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

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)*	Completion Date MM/YYYY	FIELD OF STUDY
Brown University, Providence, RI	B.S.	05/1997	Biochemistry
University of California, San Diego, CA (with Scott Emr)	Ph.D.	12/2002	Biomedical Sciences
Ludwig Institute for Cancer Research, La Jolla, CA (with Karen Oegema)	Postdoc	03/2008	Biochemistry and Cell Biology

A. Personal Statement

The major focus of my laboratory is to define the regulatory mechanisms that control organelle dynamics and membrane trafficking in neurons and other cell types. I have a strong background in the study of membrane transport, including specific training in genetics, biochemistry, and imaging. During my graduate studies, I carried out a series of genetic screens to identify new components of the secretory pathway in yeast. As a postdoctoral fellow, I applied my expertise in membrane biology to the study of ER structure and function during early *C. elegans* development. In parallel, I developed several live cell microscopy-based assays to study membrane dynamics during germline proliferation and embryogenesis. Over the last ten years, I have established my own research group and identified new, conserved components of the early secretory pathway, which play critical roles in protein secretion both in *C. elegans* and human cells. Additionally, my lab has combined *in vivo* and *in vitro* assays to define regulatory mechanisms that control vesicle transport to and from the cell surface via endosomal intermediates.

B. Positions and Honors

EMPLOYMENT:

1997-2002 Graduate Student, Biomedical Sciences Program, University of California, San Diego
2003-2008 Helen Hay Whitney Postdoctoral Fellow, Ludwig Institute for Cancer Research, La Jolla, CA
2008-2014 Assistant Professor, Biomolecular Chemistry, University of Wisconsin-Madison
2014-current Associate Professor, Biomolecular Chemistry, University of Wisconsin-Madison
2014-current Director, Molecular and Cellular Pharmacology Graduate Program
2015-current Director, UW-Madison Optical Imaging Core Facility
2016-current Affiliate, Department of Neuroscience, University of Wisconsin-Madison

HONORS, AWARDS AND OTHER PROFESSIONAL ACTIVITIES:

1997 Inducted into the Sigma Xi Honorary Society
1997 Magna cum Laude, Brown University
1997 Harvey Almy Baker Graduate Fellowship (Brown University)
1998-2002 National Cancer Institute Training Grant Award
2004-2007 Helen Hay Whitney Foundation Postdoctoral Fellowship
2006 DeLill Nasser Award for Professional Development in Genetics
2010 March of Dimes Basil O'Connor Starter Scholar Research Award

2010	Shaw Scientist Award, Greater Milwaukee Foundation
2011	Member, University of Wisconsin Comprehensive Cancer Center
2012	American Cancer Society Research Scholar Award
2012	Member, Institute for Clinical and Translational Research
2013, 2015	Ad hoc Member, NIH, Cell Biology IRG, MBPP Study Section
2013	Ad hoc Member, NIH, Molecular, Cellular, and Developmental Neuroscience IRG, SYN Study Section
2013	Review Editor, *Frontiers in Membrane Traffic*
2013	Member, Stem Cell and Regenerative Medicine Center
2013	Affiliate, Morgridge Institute for Research
2013-2017	Human Frontier Science Program (HFSP) Organization Fellowships Review Panel (Vice-Chair, 2016; Chair, 2017)
2014	Founding Director of the UW-Madison Center for Training in Pharmacology and Drug Development
2015	Steering Committee Director, UW-Madison Optical Imaging Core Facility
2015	Ad hoc Member, NIH, Cell Biology IRG, NCSD Study Section
2015	Vilas Associate Award, University of Wisconsin-Madison
2016	Vilas Faculty Early Career Investigator Award
2016-2021	T32 Training Grant (GM008688) for Predoctoral Training in Pharmacology
2016	Faculty Director, Genome Editing and Animal Models (GEAM) core facility
2017	H.I. Romnes Faculty Fellowship, University of Wisconsin-Madison
2017-2023	Standing Member, NIH, Cell Biology IRG, MBPP Study Section
2018	Advanced Scholarship, Tom Wahlig Foundation

C. Contributions to Science

1. Regulation of protein secretion and its implications in neurodegeneration and other diseases. The directed movement of proteins and membranes between different cellular locations is a fundamental process required for the proper functioning of all eukaryotic cells. Many diseases including cancer, diabetes, immune dysfunction, and neurodegenerative disorders such as hereditary spastic paraplegias can be caused by intracellular protein transport defects. My lab has discovered an essential role for Trk-fused gene (TFG; also called SPG57) in COPII-mediated protein secretion from the endoplasmic reticulum (ER). Our work over the past several years has led to a revised view of how the early secretory pathway is organized in metazoan cells, highlighting a function for SPG57/TFG in clustering COPII-coated transport carriers near their site of formation to facilitate their subsequent fusion with neighboring ER-Golgi intermediate compartments (ERGIC) upon uncoating, thereby promoting interorganellar cargo transport. In several cases, chromosomal translocation events fuse the amino-terminal portion of *TFG* to other genes, resulting in oncogenic chimeras. Our findings indicate that TFG fusion proteins interact with native TFG at the ER/ERGIC interface, which modulates secretory flow to potentially enhance cell transformation and oncogenesis. In addition, we recently discovered that mutations in *TFG* cause complicated forms of hereditary spastic paraplegia (HSP), a neurodegenerative disorder that severely limits motor control of the lower limbs. Our structural studies indicate that the mutations impair normal assembly of TFG complexes. We are now establishing tractable rodent and stem cell-based models for HSP, and we plan to develop a facile platform for identifying pharmaceutical agents to combat disease.

• Witte, K., Schuh, A.L., Hegermann, J., Sarkeshik, A., Mayers, J.R., Schwarze, K., Yates, J.R., Eimer, S., and **Audhya, A.** (2011) Mechanisms by which TFG functions in protein secretion and oncogenesis. *Nat. Cell Biol.* 13: 550-558. PMCID: PMC3311221.

• Beetz, C., Johnson, A., Schuh, A., Thakur, S., Varga, R., Fothergill, T., Hertel, N., Bomba-Warczak, E., Thiele, H., Nurnberg, G., Altmuller, J., Saxena, R., Chapman, E.R., Dent, E.W., Nurnberg, P., and **Audhya, A.** (2013) Inhibition of TFG function causes hereditary axon degeneration by impairing ER structure. *Proc. Natl. Acad. Sci. USA*. 110: 5091-5096. PMCID: PMC3612678.

• Hanna, M.G., Block, S., Frankel, E.B., Hou, F., Johnson, A., Yuan, L., Knight, G., Moresco, J.J., Yates, J.R., Ashton, R., Schekman, R., Tong, Y., and **Audhya, A.** (2017) TFG facilitates outer coat disassembly on COPII transport carriers to promote tethering and fusion with ER-Golgi intermediate compartments. *Proc. Natl. Acad. Sci. USA.* 114: E7707-E7716. PMCID: PMC5604033.

• Slosarek, E.L., Schuh, A.L., Pustova, I., Johnson, A., Bird, J., Johnson, M., Frankel, E.B., Bhattacharya, N., Hanna, M.G., Burke, J.E., Ruhl, D.A., Quinney, K., Block, S., Peotter, J.L., Chapman, E.R., Sheets, M.D.,

Butcher, S.E., Stagg, S.M., and **Audhya, A.** (2018) Pathogenic TFG mutations underlying hereditary spastic paraplegia impair secretory protein trafficking and axon fasciculation. Cell Rep. In press. PMCID: PMC6152936.

2. Mechanisms that regulate membrane scission during vesicle biogenesis and cytokinesis. Components of the ESCRT (Endosomal Sorting Complex Required for Transport) machinery have been implicated in the formation of multivesicular endosomes (MVEs), essential organelles that facilitate the turnover of integral membrane proteins and maintain cellular homeostasis at least in part by attenuating intracellular signaling mediated by cell surface receptors. MVE biogenesis involves the formation of intralumenal vesicles (ILVs), which bud away from the cytoplasm toward the endosome interior. In topologically similar processes, the ESCRT machinery also participates in membrane abscission during cytokinesis, plasma membrane repair after injury, nuclear envelope resealing after mitosis, and the formation of retroviral particles that bud from the cell surface during infection. My lab discovered the first membrane curvature sensitive component of the ESCRT machinery, which appears to play a key role in targeting ESCRT function during membrane scission events. Specifically, we have shown that a complex composed of ESCRT-II and the ESCRT-III subunit Vps20 binds selectively to membranes of elevated curvature, similar to that found at a vesicle bud neck. Our data further suggest that the ESCRT-II/Vps20 complex is mechanosensitive, binding more tightly to membranes as they become increasingly bent, which may aid in maintaining the spatial distribution of ESCRT-III to promote membrane constriction and scission. Using a combination of cryogenic electron microscopy (cryo-EM), single particle reconstruction, and molecular dynamics simulations, we have determined a pseudo-atomic structural model for the ESCRT-III complex, highlighting a spiral filament architecture composed of a series of repeating globular densities joined by flexible linkers. Our data highlight an assembly mechanism that intrinsically harnesses free energy within spiral arrays, which we speculate is released upon restructuring of the polymer. One of our current efforts is aimed at defining the regulatory components that remodel ESCRT-III filaments to facilitate membrane scission.

• Fyfe, I., Schuh, A.L., Edwardson, J.M., and **Audhya, A.** (2011) Association of the endosomal sorting complex ESCRT-II with the Vps20 subunit of ESCRT-III generates a curvature-sensitive complex capable of nucleating ESCRT-III filaments. *J. Biol. Chem*. 286: 34262-34270. PMCID: PMC3190807.

• Shen, Q., Schuh, A.L. Zheng, Y., Quinney, K., Wang, L., Hanna, M., Mitchell, J.C., Otegui, M.S., Ahlquist, P., Cui, Q., and **Audhya, A.** (2014) Mechanisms governing ESCRT-III spiral filament assembly. J. Cell Biol. 206: 763-777. PMCID: PMC4164947.

• Konig, J., Frankel, E.B., **Audhya, A**.,* and Muller-Reichert, T.* (2017) Membrane remodeling during embryonic abscission in Caenorhabditis elegans. *J. Cell Biol*. 216: 1277-1286. PMCID: PMC5412558. *Co-corresponding

• Frankel, E.B., Shankar, R., Moresco, J.J., Yates, J.R., Volkmann, N., and **Audhya, A.** (2017) Ist1 regulates ESCRT-III assembly and function during multivesicular endosome biogenesis in Caenorhabditis elegans embryos. *Nat. Commun*. 8: 1439. PMCID: PMC5682282.

3. Regulatory mechanisms that control membrane composition, structure, and dynamics. Cellular membranes are composed of numerous lipid species that function together to maintain subcellular compartmentalization and recruit downstream effector proteins. In particular, acidic phospholipids, including phosphorylated derivatives of phosphatidylinositol (PIPs) and phosphatidylserine (PS), are ideally suited to bind positively charged peptide sequences within peripheral membrane proteins, often activating these effectors to carry out their specific function(s). We have uncovered key roles for acidic phospholipids in regulating processes as diverse as cell invasion, intercellular adhesion, and intracellular protein trafficking. Our ultimate goal is to understand how specific lipid species function together with membrane binding proteins to orchestrate temporally and spatially regulated membrane remodeling events.

• Green, R., Kao, H.,* **Audhya, A**.,* Arur, S., Mayers, J.R., Fridolfsson, H., Schulman, M., Schloissnig, S., Niessen, S., Laband, K., Wang, S., Starr, D., Hyman, A., Schedl, T., Desai, A., Piano, F., Gunsalus, K.C., and Oegema, K. (2011) A high-resolution *C. elegans* essential gene network based on phenotypic profiling of a complex tissue. *Cell*. 145: 470-482. PMCID: PMC3086541. *Equal contributions

• Morrison, K., Witte, K., Mayers, J.R., Schuh, A.L., and **Audhya, A.** (2012) Role of acidic phospholipids and nucleotides in regulating membrane binding and activity of a calcium-independent phospholipase A_2 isoform. *J. Biol. Chem*. 287: 38824-38834. PMCID: PMC3493924.

• Schuh, A.L., Hanna, M., Quinney, K., Wang, L., Sarkeshik, A., Yates, J.R., and **Audhya, A.** (2015) The VPS-20 Subunit of the Endosomal Sorting Complex ESCRT-III Exhibits an Open Conformation in the Absence of Upstream Activation. *Biochem. J.* 466: 625-637. PMCID: PMC4384658.

• Johnson, A., Bhattacharya, N., Hanna, M., Pennington, J.G., Schuh, A.L., Wang, L., Otegui, M.S., Stagg, S.M., and **Audhya, A.** (2015) TFG clusters COPII-coated transport carriers and promotes early secretory pathway organization. *EMBO J.* 34: 811-827. PMCID: PMC4369316.

4. Regulatory mechanisms that govern clathrin-mediated endocytosis and endocytic recycling. A large number of cell surface molecules undergo internalization in a clathrin-dependent fashion. This process requires multiple endocytic adaptors to recognize largely distinct cargoes in a manner that relies on short signal sequences or post-translational modifications found within substrates. We discovered that the ESCRT-0 complex is recruited to sites of clathrin-mediated endocytosis at the plasma membrane, engaging ubiquitin-modified cargoes prior to their accumulation at the endosome. Our findings indicate that preassembly of ubiquitinylated cargoes with the ESCRT-0 complex at the plasma membrane enhances the efficiency of downstream sorting events in the endolysosomal system. In the absence of ubiquitin modification, many integral membrane proteins undergo endocytic recycling, an essential pathway required to maintain cell surface composition and is regulated by several Rab-type GTPases. My lab has helped to uncover new effectors of the endocytic Rab proteins, and we are currently using genetic, biochemical, and fluorescence-based functional assays for organelle remodeling to uncover the roles of these factors during development.

• Shi, A., Chen, C.C., Banerjee, R., Glodowski, D., **Audhya, A.,** Rongo, C., and Grant, B.D. (2010). EHBP-1 functions with RAB-10 during endocytic recycling in *C. elegans*. *Mol. Biol. Cell*. 21: 2930-2943. PMCID: PMC2921114.

• Mayers, J.R., Wang, L., Pramanik, J., Johnson, A., Sarkeshik, A., Wang, Y., Saengsawang, W., Yates, J.R., and **Audhya, A.** (2013) Regulation of ubiquitin-dependent cargo sorting by multiple plasma membrane endocytic adaptor proteins. *Proc. Natl. Acad. Sci. USA.* 110: 11857-11862. PMCID: PMC3718112.

• Wang, L., and **Audhya, A.** (2014) *In vivo* imaging of *C. elegans* endocytosis. *Methods*. 68: 518-528. PMCID: PMC4112158.

• Wang, L., Johnson, A., Hanna, M., and **Audhya, A**. (2016) Eps15 membrane-binding and -bending activity acts redundantly with Fcho1 during clathrin-mediated endocytosis. *Mol. Biol. Cell*. 27: 2675-2687. PMCID: PMC5007088.

5. Mechanisms that regulate post-Golgi cargo sorting and trafficking. Approximately one-third of all translated proteins in human cells are predicted to enter the secretory pathway, many of which must be accurately sorted at the Golgi apparatus to efficiently reach their final destination. Our studies have helped to identify new regulatory mechanisms that govern post-Golgi cargo transport. We have demonstrated a key role for the ESCRT-0 complex in stably associating with ubiquitin-modified cargoes and directing their incorporation into lumenal vesicles within multivesicular endosomes. Additionally, we have participated in the identification of new regulatory factors that direct the trafficking of essential cargoes, including integrin complexes, which mediate cell adhesion, and insulin, which plays critical roles in metabolic regulation. In the future, we plan to determine mechanisms by which these regulatory proteins enable cargo-selective transport toward unique secretory pathways using a combination of biochemical and high-resolution imaging approaches.

• Mayers, J.R., Fyfe, I., Schuh, A.L., Chapman, E.R., Edwardson, J.M., and **Audhya, A.** (2011) ESCRT-0 assembles as a heterotetrameric complex on membranes and binds multiple ubiquitinylated cargoes simultaneously. *J. Biol. Chem*. 286: 9636-9645. PMCID: PMC3058970.

• Wan, J., Zhu, F., Zasadil, L., Yu, J., Wang, L., Johnson, A., Berthier, E., Beebe, D.J., **Audhya, A.**, and Weaver, B.A. (2014) A Golgi localized pool of the mitotic checkpoint component Mad1 controls integrin secretion and cell migration. *Curr. Biol.* 24: 2687-2692. PMCID: PMC4254593.

• Kebede, M., Oler, A., Balloon, A.J., Johnson, A., Rabaglia, M., Stapleton, D., Schueler, K., Floyd, B., Richards, O., Raines, S., Gregg, T., Eliceiri, K., Weisshaar J., Rhodes, C., Thorstenson, C., Keller, M.P., Coon, J., **Audhya, A.**, and Attie, A.D. (2014) Sorcs1 is Required for Normal Dense Core Vesicle Biogenesis in Metabolically Stressed Beta-Cells. *J. Clin. Invest.* 124: 4240-4256. PMCID: PMC4191024.

• Takahashi, H., Mayers, J.R., Wang, L., Edwardson, J.M., and **Audhya, A.** (2015) Hrs and STAM function synergistically to bind ubiquitin-modified cargoes in vitro. *Biophys. J.* 108: 76-84. PMCID: PMC4286613.

Complete List of Published Work in PubMed:
http://www.ncbi.nlm.nih.gov/pubmed/?term=audhya+a+not+nagpure+not+chaudhury

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

Ongoing Research Support:

National Institutes of Health, NIGMS
Grant #1R01GM110567 (Anjon Audhya, PI) 3/1/2015 - 1/31/2019
Regulatory mechanisms that control vesicle secretion at the endoplasmic reticulum
The major goals of this project are to define the contributions of TFG to COPII-mediated membrane transport. **Overlap: None.**

National Institutes of Health, NIGMS
Grant #1R01GM088151 (Anjon Audhya, PI) 7/1/2010 - 3/31/2020
Molecular Mechanisms that Regulate Lysosomal Protein Transport
The major goals of this project are to determine mechanisms by which the ESCRT machinery recognizes substrates and to define mechanisms that regulate cargo entry into the ESCRT pathway. **Overlap: None.**

National Institutes of Health, NIDDK
Grant #1R01DK102948 (Anjon Audhya, co-I) 7/1/2015 - 6/30/2020
Role of Sorcs1 in Diabetes Susceptibility
The major goals of this project are to discover the role of Sorcs1 and Sortilin in insulin degradation, characterize SNPs in human *SORT1* associated with low insulin and hypercholesterolemia in an Amish population, and characterize the SNP in the pro-peptide of Sorcs1. **Overlap: None.**

Spastic Paraplegia Foundation
Grant # MSN198794 (Anjon Audhya, PI) 2/1/2017 - 1/31/2019
Axonal trafficking and organelle dynamics in hereditary spastic paraplegia
The major goals of this project are to create avenues toward the development of new therapeutic interventions for HSP. **Overlap: None.**

National Science Foundation
Grant # 1661900 (Anjon Audhya, co-PI) 7/1/2017 - 6/30/2021
Collaborative Research: Multi-scale modeling of membrane fission
The major goals of this project are to develop mathematical theory in ESCRT-III geometry and dynamics on a fixed membrane, nonlocal hydrodynamics of membrane-bound filament motion, and evolution of the fully coupled ESCRT-membrane system. **Overlap: None.**

National Institutes of Health, NIGMS
Grant #1R01GM117473 (Anjon Audhya, co-I) 9/21/2017 - 7/31/2021
Control of COPII vesicle trafficking by intracellular protein glycosylation
The major goals of this project are to dissect the functional impact of O-GlcNAc cycling on COPII vesicle trafficking, define the role of site-specific O-GlcNAcylation of Sec23A and Sec24D in human cells, and determine the contribution of COPII O-GlcNAcylation in vertebrate models of disease. **Overlap: None.**

National Institutes of Health, NIAID
Grant #2R01AI073289 (Anjon Audhya, co-I) 6/1/2018 - 5/31/2023
Biofilm Induced Extracellular Vesicle Pathogenesis
The major goal of this project is to define extracellular vesicle cargo that permits Candida to persist and disseminate from implanted medical devices, with a longer term goal of discovering targets for the development of innovative therapeutic agents. **Overlap: None.**

Completed Research Support (relevant to this project during the last 3 years):

Brain Research Foundation
Grant #BRFSG-2015-03 (Anjon Audhya, PI) 6/1/2015 - 5/31/2017
Endoplasmic reticulum structure and function in neuronal maintenance
The major goal of this seed grant was to establish physiologically relevant stem cell-based models to define the biological impact of SPG57/*TFG* mutations.

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: Randolph, Peter

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

POSITION TITLE: Postdoctoral Researcher

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
University of Virginia, Charlottesville, VA	BS	05/2005	Chemistry
University of Virginia, Charlottesville, VA	BA	05/2005	Biology
University of Virginia, Charlottesville, VA	PHD	05/2016	Chemistry

A. Personal Statement

The goal of the current application is to acquire training in multiple techniques with a special emphasis on electron microscopy. I am currently highly proficient in a variety of biochemical techniques (cloning, PCR, protein purification, kinetic assays, mass spectrometry (MALDI-TOF), multi-angle static light scattering, analytical size exclusion, crosslinking, small-angle x-ray scattering, isothermal calorimetry, analytical ultracentrifugation, molecular modelling, basic molecular dynamics simulations, electrophoretic mobility shift assays, general computer programming, and neutron scattering) with special emphasis on x-ray crystallography. I feel that x-ray crystallography and electron microscopy are extremely complimentary techniques, allowing structure determination from small molecules to large macromolecular complexes. I have been interested in Structural Biology for many years, graduated from the University of Virginia as an undergraduate with a double major in Biology and Biochemistry. I returned to the University of Virginia to complete my PhD under the tutelage of Dr. Cameron Mura, learning x-ray crystallography from established experts including Drs. Duilio Cascio and Michael Sawaya from the University of California, LA. I hope to combine my knowledge of biophysical techniques, x-ray crystallography, and electron microscopy to become a primary investigator and research leader in the field of structural biology.

B. Positions and Honors

Positions and Employment

2005 - 2007 Radiation Safety Technician, University of Virginia, Office of Environmental Health and Safety, Charlottesville, VA

2007 - 2010 Fire Marshall, University of Virginia, Office of Environmental Health and Safety, Charlottesville, VA

2010 - 2011 Teaching Assistant, University of Virginia , Department of Chemistry, Charlottesville, VA

2010 - 2016 Graduate Researcher, University of Virginia, Department of Chemistry, Charlottesville, VA

2016 - Postdoctoral Researcher, Florida State University, Institute of Molecular Biophysics, Tallahassee, FL

Other Experience and Professional Memberships

2012 - Member, American Association for the Advancement of Science (AAAS)

2014 - Member, Biophysics Society

2014 - Member, American Crystallographic Association

Honors

2012 - 2014 Biophysics Training Grant, NIH

2013 Robert J. Huskey Travel Fellowship, University of Virgina

2013 Invited Speaker, Symposium on RNA X: RNA Tool and Target
2013 Invited Participant - 4th Neutrons in Structural Biology Workshop, Oak Ridge National Laboratory
2013 Robert J. Huskey Graduate School Research Exhibition, 2nd Place, University of Virginia
2014 Pittsburgh Diffraction Society Travel Grant, Pittsburgh Diffraction Society

C. Contribution to Science

1. During my graduate work I examined the ubiquitous RNA-binding Sm proteins. While both the eukaryal and bacterial branch of Sm proteins are well characterized, I focused on the archaeal branch. Sm-like archaeal proteins (SmAPs) provided a unique opportunity, effectively sharing traits from both the eukaryal and bacterial Sm proteins. My research into SmAPs bridged the gap between archaea and eukarya, and demonstrated a putative novel mode of U-rich RNA binding. In addition, I solved the first Sm structure to contain an octameric fold within the asymmetry unit, and further combines to form a large 'cage' structure consisting of six octamers, opening up the possibility of larger Sm oligomers.

    a. Stanek KA, Patterson-West J, Randolph PS, Mura C. Crystal structure and RNA-binding properties of an Hfq homolog from the deep-branching Aquificae: conservation of the lateral RNA-binding mode. Acta Crystallogr D Struct Biol. 2017 Apr 1;73(Pt 4):294-315. PubMed PMID: 28375142; PubMed Central PMCID: PMC5379935.
    b. Mura C, Randolph PS, Patterson J, Cozen AE. Archaeal and eukaryotic homologs of Hfq: A structural and evolutionary perspective on Sm function. RNA Biol. 2013 Apr;10(4):636-51. PubMed PMID: 23579284; PubMed Central PMCID: PMC3710371.

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

Completed Research Support

T32 GM080186-05
BUSHWELLER, JOHN Hackett (PI)
07/01/08-06/30/13
Training in Molecular Biophysics
Role: TA

T32 GM080186-04
BUSHWELLER, JOHN Hackett (PI)
07/01/08-06/30/13
Training in Molecular Biophysics
Role: TA