BIOGRAPHICAL SKETCH

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NAME: Lauren Parker Jackson

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

POSITION TITLE: Assistant Professor of Biological Sciences & Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Vanderbilt University	B.S.	05/2003	Chemistry
MRC Laboratory of Molecular Biology & Trinity College, University of Cambridge	Ph.D.	01/2008	Molecular Biology/ Structural Biology
Cambridge Institute for Medical Research	Postdoctoral	11/2013	Biochemistry

A. Personal Statement

The goal of my laboratory is to understand the cellular roles of important protein complexes that initiate cellular trafficking pathways by forming coats around vesicles or tubules at specific membranes. We focus on the Assembly Polypeptide (AP) family and retromer coat complexes and the roles they undertake in fundamental cell biology and human disease. Each coat functions as a "hub" to coordinate large protein networks that drive regulated formation of vesicles or tubules at precise membrane locations. We primarily use structural, biochemical, and biophysical methods to address at the molecular level how coats interact with protein and lipid partners to regulate coat assembly and recognize important cargoes. We use our mechanistic data to address functional relevance of coat protein complexes in cultured cell lines or in model systems, including budding yeast. Ultimately, we aim to characterize molecular mechanisms of coat protein assembly and regulation. Our goal is to understand what pathways coats influence and how coats drive human disease, including cancers and brain disorders. Over the past five years, we have expanded our repertoire beyond my graduate and postgraduate training to include cryo-electron microscopy, cryo-electron tomography, and immunofluorescence imaging. The lab has determined and published multiple new structures of retromer, COPI, and AP-4 coat components that provide insight into coat assembly and regulation at membranes.

A. Chandra M†, Kendall AK, and **Jackson LP**†. (2021). Towards understanding the molecular role of SNX27/retromer in human health and disease. *Frontiers Cell & Developmental Biology.* doi: 10.3389/fcell.2021.642378. (†corresponding authors. Online ahead of print). PMCID: PMC8083963.

B. Chandra M†, Kendall AK, and **Jackson LP**†. (2020). Unveiling the cryo-EM structure of Retromer. *Biochemical Society Transactions* 48(5): 2261-2272. (†corresponding authors). PMCID not available.

C. Frazier MN and **Jackson LP**. (2017). Spotlight: Watching real-time endocytosis in living cells. *Journal of Cell Biology* 216(1):9-11. PMCID not available.

D. **Jackson LP**. (2014). Structure and mechanism of COPI vesicle biogenesis. *Curr Opin Cell Biol* **29C**, 67-73. PMCID not available.

B. Employment, Honors, & Service

Employment

2014-present Assistant Professor, Dept. of Biological Sciences, Vanderbilt University, Nashville, TN, USA
2016-present Assistant Professor, Dept. of Biochemistry, Vanderbilt University School of Medicine
2009-2013 Postdoctoral Research Associate, Cambridge Institute for Medical Research, Cambridge, UK
2009-2013 Supervisor, Natural Sciences Part IA, Jesus College, Cambridge, UK (teaching experience)
2007-2009 Junior Consultant, The Boston Consulting Group, London, UK

Honors

2019 Nordhaus Award for Excellence in Undergraduate Teaching, Vanderbilt College of Arts & Sciences
2017 Vanderbilt Faculty Cutting-Edge Flexner Discovery Lecture
2016 Pew Biomedical Scholars Award
2016 Provost Research Studio for faculty development, Vanderbilt University
2016 Littlejohn Faculty Fellow, Vanderbilt Undergraduate Summer Research Program
2013 Gordon Research Conference travel award (Molecular Membrane Biology)
2012 Keystone Symposia Future of Science Fund Scholarship (Structural Biology of Cellular Processes)
2011 Protein Society Young Investigator Travel Grant/Finn Wold Travel Award
2010 Gordon Research Conference travel award (Lysosomes and Endocytosis)
2004 Academy of Achievement International Achievement Summit, Chicago, IL
2003 MRC Laboratory of Molecular Biology Student Scholarship
2003 Trinity College Honorary External Research Studentship
2003 National Science Foundation Fellowship (declined)
2003 Gates Cambridge Scholarship (declined)
2003 Founder's Medalist, College of Arts & Science, Vanderbilt University
2003 Phi Beta Kappa, Vanderbilt University
2003 Joel Tellinghuisen Award for Undergraduate Research, Phi Beta Kappa, Vanderbilt University
2003 Outstanding Senior in Chemistry, Vanderbilt University Dept. of Chemistry
2003 Donald E Pearson Award for Undergraduate Research, Vanderbilt University Dept. of Chemistry

Professional Service

Discussion leader, GRC Lysosomes & Endocytosis, June 2020 (canceled due to COVID-19)
Editorial board member, *Traffic*, 2017-2020
Guest Editor, *Traffic* review series ("Traffic at Atomic Resolution"), 2019
Member, Planning Committee, 2018 Pew Annual Meeting
Chair, Science Session I, 2018 Pew Annual Meeting
F1000 contributing faculty member, Cell Signaling & Trafficking Structures, 2018-2020

Reviewing activity

- *Ad hoc* grant reviewer for NIH Membrane Biology & Protein Processing Study Section; Wellcome Trust (UK); Medical Research Council (UK), Deutsche Forschungsgemeinschaft (DFG)
- *Ad hoc* journal reviewer for *Nature Structural & Molecular Biology, eLife, Nature Communications, Nature Chemical Biology, Proc Natl Acad Sci U S A*, *Journal of Cell Biology, EMBO Journal, Journal of Biological Chemistry, Structure, Traffic, Wellcome Open, Trends in Biochemical Sciences;* book chapter in "Biomolecular and Bioanalytical Techniques: Theory, Methodology and Applications", Wiley (UK)
- *Ad hoc* reviewer for Stanford Synchrotron Radiation Light Source (SSRL)

Professional memberships

Member, American Society for Cell Biology, 2015-present
Member, Biophysical Society, 2017-present

C. Contributions to Science

AP-4 coat structure and assembly

AP-4 is a member of the Assembly Polypeptide (AP) protein family of vesicular coat proteins. Most APs associate with a scaffold like clathrin, while AP-4 appears to lack a scaffold. How AP-4 coats assemble to engage cargo and promote vesicle formation thus remains a major question in the field. We have used a variety of tools and techniques to gain understanding of AP-4 structure and evolution. My group uncovered how AP-4 interacts with its major accessory protein, tepsin, by identifying and testing one of the first two AP-4 binding motifs. We determined X-ray crystal structures of both tepsin structured domains and established the evolutionary history of tepsin. We will further explore how divergence away from related family members likely contributes to a different biological function for tepsin. We also explored genetic variation in AP-4 across human populations, and we developed modeling tools to predict pathogenicity of newly identified AP-4 mutations in patients. Finally, we have tested newly identified AP-4 coat components, including the RUSC proteins, in collaborative projects.

A. Gadbery JE*, Abraham A*, Needle C, Moth C, Sheehan J, Capra JA, **Jackson LP**. (2020). Integrating Structural and Evolutionary Data to Interpret Genetic Variation and Pathogenicity in Adaptor Protein Complex 4 (AP-4). *Protein Science* 29 (6):1535-1549. (*joint first authors) PMCID: PMC7255511.

B. Davies AK, Itzhak DN, Edgar JR, Archuleta TL, Hirst J, **Jackson LP**, Robinson MS, and Borner GHH. (2018). AP-4 vesicles contribute to spatial control of autophagy via RUSC-dependent peripheral delivery of ATG9A. *Nature Communications* 9(1): 3958. PMCID: PMC6160451.

C. Archuleta TL*, Frazier MN*, Monken A, Kendall AK, Harp J, McCoy AJ, Creanza N, and **Jackson LP**. (2017). Structure and evolution of ENTH and VHS/ENTH-like domains in tepsin. *Traffic* 18, 590-603. (*joint first authors), PMCID: PMC5567745.

D. Frazier MN, Davies AK, Voehler M, Kendall AK, Borner GHH, Chazin WJ, Robinson MS, and **Jackson LP**. (2016). Molecular basis for the interaction between Adaptor Protein Complex 4 (AP4) β4 and its accessory protein, tepsin. *Traffic* 17, 400-415. PMCID: PMC4805503.

Assembly & regulation of metazoan retromer coats

On endosomes, metazoan retromer (VPS26/VPS35/VPS29 subunits) sorts cargoes to multiple destinations, including the plasma membrane and *trans*-Golgi network (TGN). My interests in retromer began as a postdoctoral fellow. I established the biochemical basis for how the retromer VPS29 subunit binds a protein called VARP, which likely regulates retromer function in cells. A major question in the field is how retromer assembles to sort different protein cargoes to multiple destinations from a common origin. My laboratory determined the first single particle cryo-EM structures of murine retromer. These structures revealed the presence of multiple oligomers *in vitro*; retromer assembles into dimers, tetramers, and elongated chain structures linked by VPS35 and VPS26 homodimers. Sub-structure determination allowed us to identify a key VPS35 dimer assembly interface; we tested the interface biochemically and demonstrated likely conservation across eukaryotes. These structures raise important questions about whether retromer may serve as an adaptable scaffold to accommodate different sorting nexin protein partners in metazoans, an idea we will test in our proposal. We are also working to improve structural models using cryo-EM, since retromer is a challenging target. Finally, we continue to pursue structures of retromer with small molecules to establish whether retromer is a suitable therapeutic target.

A. Kendall AK, Xie B, Xu P, Wang J, Burcham B, Frazier MN, Binshtein E, Wei H, Graham TR, Nakagawa T, **Jackson LP**. (2020). Mammalian retromer is an adaptable scaffold for cargo sorting from endosomes. *Structure* 28, 393-405. PMCID: PMC7145723. (Featured on cover and in Preview article)

B. Kai-En Chen, Qian Guo, Yi Cui, Amy K. Kendall, Joanna Sacharz, Timothy A. Hill, Ryan J. Hall, Suzanne J. Norwood, Natalya Leneva, Boyang Xie, Zhe Yang, Rajesh Ghai, Hiroaki Suga, David Fairlie, David A. Stroud, **Jackson LP**, Rohan D. Teasdale, Toby Passioura, Brett M. Collins. (2020). *De novo* macrocyclic peptides for inhibiting, stabilising and probing the function of the Retromer endosomal trafficking complex. *bioRxiv* 2020. 12.03.410779; doi: https://doi.org/10.1101/2020.12.03.410779. (In revision.)

C. Crawley-Snowdon H, Yang J-C, Zaccai NR, Davis L, Wartosch L, Herman EK, Bright NA, Swarbrick JS, Collins BM, **Jackson LP**, Seaman MNJS, Luzio JP, Dacks JB†, Neuhaus D†, Owen DJ†. (2020). Mechanism and evolution of the Zn-fingernail required for interaction of VARP with VPS29. *Nature Communications* 11(1):5031. PMCID: PMC7539009.

D. Hesketh GG*, Pérez-Dorado I*, **Jackson LP**, Wartosch L, Schäfer IB, Gray SR, McCoy AJ, Zeldin OB, Garman EF, Harbour ME, Evans PR, Seaman MN, Luzio JP, Owen DJ. (2014). VARP is Recruited on to Endosomes by Direct Interaction with Retromer, Where Together They Function in Export to the Cell Surface. *Developmental Cell* 29(5):591-606. PMCID: PMC4059916.

Assembly & regulation of COPI coats

Essential COPI coats are conserved from yeast to humans. As a postdoc, I provided the first molecular data on how the COPI coat interacts with any of its important cargoes in the retrograde pathway. X-ray structures elucidated how two COPI subunits (α-COP and β'-COP) interact with dilysine motifs found in retrograde cargoes; these data implied COPI coats assemble differently from clathrin coats, which was later demonstrated by cryo-ET. My independent work has focused more broadly on the role of β'-COP within COPI coats. In collaboration with Todd Graham's lab, we uncovered the biochemical basis for recognition of a ubiquitinated SNARE protein by β'-COP. This work highlighted how ubiquitin can act as an important retrieval signal for a SNARE. In ongoing work, we are investigating regulation of COPI coats by ArfGAP proteins by focusing on the yeast ArfGAP, Glo3. We determined the first X-ray crystal structure of the Glo3 GAP domain, and we combined structural and conservation data to propose how Glo3 GAP domain binds its small GTPase (Arf1) in the context of membrane-assembled COPI coats. These studies set the stage for our ongoing work exploring how β'-COP directly engages Glo3, as outlined in the proposal.

A. Xie B, Jung C, Chandra M, Engel A, Kendall AK, and **Jackson LP**. (2021). The Glo3 GAP crystal structure supports the molecular niche model for ArfGAPs in COPI coats. *Advances in Biological Regulation* 79:100781. PMCID: PMC7920988.

B. Xu P, Hankins HM, Macdonald C, Erlinger SJ, Frazier MN, Diab NS, Piper RC, **Jackson LP**, MacGurn JA, and Graham TR. (2017). COPI mediates recycling of an exocytic SNARE from endosomes by recognition of a ubiquitin sorting signal. *eLife* 2017; 6:e28342. DOI:10.7554/eLife.28342. PMCID: PMC5663479.

C. **Jackson LP**†, Lewis M, Kent HM, Edeling MA, Evans PR, Duden R, and Owen DJ†. (2012). Molecular basis for recognition of dilysine trafficking motifs by COPI. *Dev Cell* **23**, 1-8 (†corresponding authors), PMCID: PMC3521961.

Cargo recognition in clathrin coated vesicles (postdoctoral & graduate work)

The identification and sorting of protein cargoes to specific destinations lie at the heart of membrane trafficking. Clathrin-mediated endocytosis at the plasma membrane has long served as a paradigm for understanding coated vesicle formation. My postdoctoral work revealed how AP-2 binds short linear motifs in cargo after undergoing a large conformational change upon membrane recruitment by phosphoinositides. Subsequent work by other groups showed how this conformational change is conserved in related coats (AP-1, COPI). A second major question was how SNARE proteins are sorted as cargo back to their steady-state destination following a fusion event. Our work on the lysosomal SNARE protein, VAMP7, provided one of the first two structural examples of how coats package SNAREs into forming vesicles in a non-competitive way. Instead of linear motifs, SNAREs instead use folded structural domains to interact specifically with a single adaptor protein, and loss of important residues in these domains have important implications for mis-sorting.

A. **Jackson LP**†, Kümmel D†, Reinisch K, and Owen DJ. (2012). Structures and mechanisms of vesicle coat components and multisubunit tethering complexes. *Current Opinion in Cell Biology* 24(4):475-83. (†corresponding authors). PMCID: PMC3425711

B. **Jackson LP***, Kelly BT*, McCoy AJ, Gaffry T, James LC, Collins BM, Höning S, Evans PR, Owen DJ. (2010). A large scale conformational change couples membrane recruitment to cargo binding in the AP2 clathrin adaptor complex. *Cell* 141, 1220-29, (*joint first authors), PMCID: PMC3655264.

C. Pryor PR, **Jackson LP**, Gray SR, Edeling MA, Thompson A, Sanderson CM, Evans PR, Owen DJ, Luzio JP. (2008). Molecular basis for the sorting of the SNARE VAMP7 into endocytic clathrin-coated vesicles by the ArfGAP Hrb. *Cell* 134, 817-27, PMCID: PMC2648964.

Structural studies of filamentous plant viruses (undergraduate work)
Filamentous plant viruses are important models for understanding helical virus assembly and in agricultural disease; potyviruses alone account for more than half the viral crop damage world-wide. Because of their filamentous nature, these viruses do not crystallize. As an undergraduate, I helped develop methods for making filamentous virus samples of the potexvirus, potato virus X (PVX), and the potyvirus, wheat streak mosaic virus (WSMV). Our fiber diffraction data on PVX provided the first estimates of helical symmetry for the virus, while our work on WSMV was one of the first examples of potyvirus fiber diffraction. Subsequently, the combination of structural techniques like EM and STEM was used together with our fiber diffraction data to produce a more detailed analysis of PVX structure.

A. **Parker L**, Kendall A, Berger, PH, Shiel, PJ, and Stubbs, G. (2005). Wheat streak mosaic virus— Structural parameters for a *Potyvirus*. *Virology* 340, 64-69. (Cover article)

B. Stubbs G, **Parker L**, Junn J, and Kendall, A. (2005). Flexible filamentous virus structures from fiber diffraction. *Fiber Diffraction Review* 13, 38-42.

C. **Parker L**, Kendall A, and Stubbs, G. (2002). Surface Features of Potato Virus X from Fiber Diffraction. *Virology* 300, 291-29. (Cover article)

Full publication list: https://www.ncbi.nlm.nih.gov/myncbi/10O8lSLY-fBAo/bibliography/public/

D. Research Support

Current funding

1. NIH/NIGMS R35GM119525 09/01/2016 – 05/31/2022 (NCE) Role: PI
"Molecular mechanisms of coat protein assembly and regulation in membrane trafficking"

2. NIH/NIGMS 3R35GM119525-05S1 06/01/2020 – 05/31/2021 Role: PI
"The molecular role of tepsin in membrane trafficking pathways"
This is a supplement to support award 5R35GM119525-05

3. NIH/NCI 1R01 CA224188-01A1 07/01/2020-06/30/2025 Role: Collaborator
(PI: Yashi Ahmed, Dartmouth; Vanderbilt PI: Ethan Lee)
"Inhibition of the Wnt Receptor Complex by the Tumor Suppressor Adenomatous Polyposis Coli"

4. NIH/NIGMS R01GM1184532 07/01/2016-04/30/2021 (NCE) Role: Collaborator
(PI: Todd Graham)
"Mechanisms of protein transport between Golgi and endosomes"

Completed support

1. Pew Charitable Trusts, Pew Scholars Award 08/01/2016 – 07/31/2020 Role: PI
"Coat protein function in membrane trafficking and human disease"

2. NIH/NIGMS 3R35GM119525-01S1 09/1/2016-5/31/2017 Role: PI
"Molecular mechanisms of coat protein assembly and regulation in membrane trafficking
This was a diversity supplement to support award 5R35GM119525-03.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Ford, Marijn Gerard Johannes

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

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Cambridge, Cambridge, United Kingdom	BA	06/1998	Natural Sciences
University of Cambridge, Cambridge, United Kingdom	PHD	02/2003	Molecular Biology
Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom	Postdoctoral Fellow	09/2003	Molecular Biology
Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom	Postdoctoral Fellow	06/2007	Molecular Biology
University of California, Davis, Davis, CA	Postdoctoral Fellow	12/2012	Molecular Biology

A. Personal Statement

I am interested in the fundamental question of how the cell controls the morphology and structure of its membranes. My training, in the laboratories of Doctors McMahon and Evans at the Medical Research Council Laboratory of Molecular Biology in Cambridge, UK, provided a comprehensive grounding in structural, biophysical and biochemical techniques. My work produced several seminal structures that were instrumental in developing the field examining the molecular mechanisms of membrane curvature generation. I gained further expertise in yeast cell biology and high-throughput genetics in Professor Nunnari's laboratory at the University of California, Davis, where I had an opportunity to work on several yeast and mammalian DSPs. During my time in the Nunnari laboratory, I overcame a key obstacle in the DSP field by solving and publishing the long-awaited crystal structure of Dynamin 1, at the time the first structure of intact dynamin to be determined. This work allowed us to present a model for DSP assembly and membrane scission that was highly influential in the field. Since I started my laboratory at the University of Pittsburgh School of Medicine, we have used crystallography and cryo-electron microscopy (cryoEM) to generate a complete structural model for the poorly characterized fungal, endosomal DSP Vps1. Assembled Vps1 has a markedly different architecture compared to assembled dynamin. Hence, comparison allowed us to generate novel insights into the mechanisms of assembly of all DSPs. Our focus then expanded to other endosomal membrane remodelers. Using cryoEM, we determined the high-resolution single particle structure of the SNX-BAR Mvp1. Unexpectedly, full-length SNX-BAR Mvp1 is tetrameric, with the lipid binding pockets and interfaces of its PX and BAR domains sequestered into the interior of a tight embrace, which precludes lipid binding and remodeling. Tetramerization is due to the presence of a low-complexity N-terminal extension on Mvp1 and its removal renders it dimeric. Curiously, several of the SNX-BAR proteins engaged in retromer-mediated retrograde trafficking from the endosome, in both yeast and mammalian cells, have uncharacterized N-terminal extensions also. Hence, we are currently examining whether tetramerization is a novel and widely conserved regulatory mechanism for a wide family of endosomal remodeling proteins, many of which have disease associations. A second direction of my lab is to examine the molecular mechanisms of TORC1 signaling and stress responses using yeast as a model. Recent work has demonstrated that this signaling is tightly interwoven with endosomal dynamics and our immediate direction is to identify and elaborate understanding of these molecular connections. To summarize, our laboratory has a

demonstrated track-record of high-quality work on DSPs, SNX-BARs and other proteins that function in membrane remodeling.

Since starting my independent research career, I have trained a postdoctoral fellow, several undergraduate and masters' students and two research. I am also a member of the training faculty for the Cell Biology and Molecular Physiology program at the University of Pittsburgh School of Medicine and currently have a graduate student from this program. She has just been awarded a position as a Trainee on the National Research Service Award (NRSA) entitled, "Interinstitutional Program in Cell Biology and Molecular Biology: A Graduate Training Path to Promote Traditional and Non-Traditional Professional Outcomes" Grant #1T32GM133353-01A1. I am also currently serving on four thesis committees within the School of Medicine, in addition to serving as a member of the teaching faculty for several courses for several graduate programs. All my trainees – including undergraduate – receive individual projects to foster ownership, to encourage the highest standards of rigor and excellence, and to ensure the training is accompanied by a sense of rewarding accomplishment. I meet with all my trainees for detailed discussions about ongoing work, often more than once per week. In these, we discuss raw data, work on rigorous and thorough experimental planning, appropriate statistical analyses and design and review goals and targets for individual projects. We also have more formal lab meetings, where trainees can develop scientific communication and presentation skills. Our work is with genetically modified yeast strains and purified proteins for structural characterization, so a continued emphasis is on extensive validation of our reagents and preparations. All my trainees have generated high quality and rigorously validated data that have been used for grants and publications. I have encouraged my graduate student and postdoctoral fellow to present as much as possible and have sent my graduate student to conferences right from the outset of her time in the lab. She will exercise her scientific writing skills by drafting her papers as well as a critical review of her field of research. I am passionate about mentoring and training and strive to create a supportive, welcoming and fun research and work environment for all members of the lab.

B. Positions and Honors

Positions and Employment

2003 - 2003	Postdoctoral Research Associate, Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom
2003 - 2006	Research Fellow, Downing College, University of Cambridge, Cambridge, United Kingdom
2006 - 2007	Postdoctoral Research Associate, Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom
2007 - 2012	Postdoctoral Research Scholar, University of California, Davis, Davis, CA
2012 - 2013	Assistant Project Scientist, University of California, Davis, Davis, CA
2013 - 2020	Assistant Professor, University of Pittsburgh, Pittsburgh, PA
2020 -	Associate Professor, University of Pittsburgh, Pittsburgh, PA

Other Experience and Professional Memberships

2016 -	Institutional Biosafety Committee, University of Pittsburgh
2018 -	Annual Pittsburgh Symposium on Intracellular Membrane Traffic Committee
2018 -	American Society for Cell Biology

Grant Review and Editing

2017 -	Ad-hoc grant review and invited editor

Honors

2002	Max Perutz Student Prize for outstanding research carried out during a Ph.D., Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom
2003	Research Fellowship Competition, Downing College, University of Cambridge, Cambridge, United Kingdom
2006	Election to full Fellowship, Downing College, University of Cambridge, Cambridge, United Kingdom

C. Contribution to Science

1. During my doctoral research at the Medical Research Council Laboratory of Molecular Biology in Cambridge, UK, I was interested in membrane recruitment of early endocytic accessory proteins in the nerve terminal. Initial work focused on AP180, an adaptor protein that was, at the time, believed to regulate the size of endocytic vesicles. Our work demonstrated that the N-terminal domain interacts with phosphorylated phosphoinositides. AP180 is a neuron-enriched protein with a ubiquitous homolog, CALM. We determined the structure of the equivalent N-terminal domain from CALM, in the absence and presence of several lipid headgroups. The domain has a novel all-helical fold and was named the ANTH (AP180 N-terminal Homology) domain.

   Our work also demonstrated that full length AP180 recruits clathrin to lipid monolayers and stimulates its assembly into flat lattices. The C-terminal domain of AP180 was demonstrated to contain numerous clathrin interaction motifs and the work resulted in a model that simple cross-linking of clathrin triskelia by AP180 overcomes any activation barriers to lattice formation. This work resulted in development of a tool (the AP180 C-terminal domain) that, when transfected into several eukaryotic cells, was particularly effective in blocking clathrin-mediated endocytosis. Over the years, 100s of labs have requested and used this tool.

   a. **Ford MG**, Pearse BM, Higgins MK, Vallis Y, Owen DJ, Gibson A, Hopkins CR, Evans PR, McMahon HT. Simultaneous binding of PtdIns(4,5)P2 and clathrin by AP180 in the nucleation of clathrin lattices on membranes. Science. 2001. DOI: 10.1126/science.291.5506.1051. PMID: 11161218.

2. Further work during my doctoral research demonstrated that the N-terminal domain of an additional endocytic accessory, epsin, also interacts with membranes. Structure determination of the epsin N-terminal domain in complex with inositol-1,4,5-trisphosphate showed that the unstructured N-terminal 15 residues of the domain folds into a new and amphipathic helix, named alpha0, in the presence of the headgroup or the corresponding lipid. The crystal lattice revealed an antiparallel tetramerisation around this new helix but we were unable to demonstrate multimerization *in vitro*. Instead, we discovered that the helix, on lipid binding, inserts into the proximal leaflet of the membrane and imparts membrane curvature. This was one of the first demonstrations of membrane deformation by a protein domain and several other proteins operating at several membranes within the endomembrane system were subsequently demonstrated to also fold an N-terminal amphipathic alpha helix on ligand biding.

   a. **Ford MG**, Mills IG, Peter BJ, Vallis Y, Praefcke GJ, Evans PR, McMahon HT. Curvature of clathrin-coated pits driven by epsin. Nature. 2002. DOI: 10.1038/nature01020. PMID: 12353027.

3. Work during my tenure as a Research Fellow at Downing College, University of Cambridge, focused on structural, biophysical and biochemical characterization of the appendage domains of the AP2 adaptor protein complex. The work aimed to determine the mechanism whereby so many endocytic proteins interact with a relatively small domain. We showed that accessory proteins interact with the alpha-appendage via short peptide motifs embedded within typically unstructured regions of the accessory proteins. We determined the structure of the alpha-appendage in complex with a peptide bound to the previously identified "platform" subdomain of the alpha-appendage. We obtained additional co-crystals of the alpha-appendage bound to a different high-affinity peptide ligand and were surprised to discover an interaction with the "sandwich" subdomain. As some accessory proteins have several embedded alpha-appendage-interacting motifs, we were able to explain how individual weak interactions could generate binding of extreme avidity. We developed a hub-model for alpha-appendage interaction patterns that sought to explain directionality through the early stages of assembly of the endocytic machine.

   Further work resulted in the determination of the structures of the beta2-appendage with various peptides. The beta2-appendage also has separate peptide binding sites on its platform and sandwich subdomains though the mechanisms of interaction are different than the in the case of the alpha-appendage. The beta-arrestin C-terminal peptide binds to the platform subdomain as an alpha helix, whereas binding to the sandwich subdomain relies on two hydrophobic residues binding into shallow pockets. As the accessory proteins interacting with the beta2-appendage are different to those interacting with the alpha-appendage, we could explain how some accessory proteins persist into the fully assembled clathrin-coated vesicle.

This work resulted in 3 joint first author publications.

a. Olesen LE*, **Ford MG***, Schmid EM*, Vallis Y, Babu MM, Li PH, Mills IG, McMahon HT, Praefcke GJ. Solitary and repetitive binding motifs for the AP2 complex alpha-appendage in amphiphysin and other accessory proteins. J Biol Chem. 2008. DOI: 10.1074/jbc.M708621200. PMID: 17986441.

b. Schmid EM*, **Ford MG***, Burtey A, Praefcke GJ, Peak-Chew SY, Mills IG, Benmerah A, McMahon HT. Role of the AP2 beta-appendage hub in recruiting partners for clathrin-coated vesicle assembly. PLoS Biol. 2006. DOI: 10.1371/journal.pbio.0040262. PMID: 16903783; PMCID: PMC1540706.

c. Praefcke GJ*, **Ford MG***, Schmid EM, Olesen LE, Gallop JL, Peak-Chew SY, Vallis Y, Babu MM, Mills IG, McMahon HT. Evolving nature of the AP2 alpha-appendage hub during clathrin-coated vesicle endocytosis. EMBO J. 2004. DOI: 10.1038/sj.emboj.7600445. PMID: 15496985; PMCID: PMC526462.

4. My work at the University of California, Davis, was directed at obtaining structural information on a member of the DSP family. Dynamin, the most extensively characterized DSP, catalyzes the scission step of clathrin-mediated endocytosis. Other members of the DSP family catalyze membrane remodeling events at other membranes throughout the cell, including the mitochondrion and endosome. In the history of dynamin and DSP research, several models have been presented for its mechanism of action in vesicle scission.

   We reasoned that DSP self-assembly into helices precluded successful crystallization. We therefore screened conserved residues within the DSP family for those that might specifically disrupt self-assembly. We therefore developed a simple optical assay using the yeast mitochondrial division dynamin, Dnm1, and the highly dynamic nature of the yeast mitochondrial network. In the absence of functional Dnm1, the mitochondrial network extensively fuses and interconnects. We identified a point mutant that is unable to rescue the mitochondrial network but still localized to the network. Biochemical, biophysical and EM analyses confirmed that the point mutant, translated into dynamin itself, is an assembly-deficient dimer. This mutant crystallized and we determined the structure of nearly full-length dynamin lacking only the C-terminal PRD domain, which is unstructured (dynamin G397D). The structure revealed the mechanism of dynamin self-assembly. Dynamin dimerizes via its “Interface 2” and higher order assembly depends on Interfaces 1 and 3. The G397D mutation lies in a tight loop that forms Interface 3. The crystal lattice was built on extended linear filaments. However, interface 3 was crowded and disordered in the structure. We therefore speculate that the G to D mutation prevents assembly into helical filaments.

   To dock the crystal structure into existing cryoEM reconstructions of GMPPCP-bound dynamin, we proposed a significant conformational change occurs between the dynamin GTPase and BSEs, in a GTP hydrolysis-dependent manner. Strikingly, this conformational change explained the molecular basis for the two temperature-sensitive *shibire* mutations, as well as the so-called *sushi* (suppressor of *shibire*) mutants.

   In my own laboratory at the Department of Cell Biology at the University of Pittsburgh School of Medicine, we continued our study on the mechanisms of membrane remodeling by DSPs, using the poorly characterized fungal endosomal DSP Vps1 as a model. Using crystallography and cryoEM, we determined the structures of domains of Vps1 in various nucleotide states and a structure of the full-length Vps1 helical assembly bound to the non-hydrolyzable GTP analog GMPPCP. We developed a complete pseudo-atomic structure of the Vps1 assembly and discovered important differences in the assembly interfaces when compared to dynamin and other DSP structures. The Vps1 helical assembly is more open and flexible and is stabilized by novel interfaces not observed in other DSPs. Biochemically, Vps1 does not share the robust assembly-stimulated GTP hydrolysis of dynamin and we propose this may be an adaptation for stabilizing tubulated membrane at the endosome rather than catalyzing scission.

   a. **Ford MG**, Jenni S, Nunnari J. The crystal structure of dynamin. Nature. 2011. DOI: 10.1038/nature10441. PMID: 21927001; PMCID: PMC4075756.

   b. Antonny B, Burd C, De Camilli P, Chen E, Daumke O, Faelber K, **Ford MGJ**, Frolov VA, Frost A, Hinshaw JE, Kirchhausen T, Kozlov MM, Lenz M, Low HH, McMahon H, Merrifield C, Pollard TD, Robinson PJ, Roux A, Schmid S. Membrane fission by dynamin: what we know and what we need to know. EMBO J. 2016. DOI: 10.15252/embj.201694613. PMID: 27670760; PMCID: PMC5090216.

c. Varlakhanova NV, Alvarez FJ, Brady TM, Tornabene BA, Hosford CJ, Chappie JS, Zhang P, **Ford MGJ**. Structures of the fungal dynamin related protein Vps1 reveal a unique, open helical architecture. J. Cell Biol. 2018. DOI: 10.1083/jcb.201712021. PMID: 30087125; PMCID: PMC6168280.
d. **Ford MGJ** & Chappie, JS. Invited review article. The Structural Biology of the Dynamin-Related Proteins: New Insights into a Diverse, Multi-Talented Family. Traffic. 2019. DOI: 10.1111/tra.12676. PMID: 31298797; PMCID (available 2020-10-01): PMC6876869.
e. Tornabene BA, Varlakhanova NV, Hosford CJ, Chappie JS, **Ford MGJ**. Structural and Functional Characterization of the Dominant Negative P-Loop Lysine Mutation in the Dynamin Superfamily Protein Vps1. Protein Sci. 2020. DOI: 10.1002/pro.3830. PMID: 31981262; PMCID (available 2021-06-01): PMC7255512.

5. More recently, our interests have branched into the characterization of SNX-BAR proteins, which are key membrane remodeling machines in all eukaryotic cells. Mammalian cells have 12. We determined the cryoEM structure of the yeast SNX-BAR protein Mvp1, which revealed a novel and unexpected tetrameric configuration: the lipid binding pockets and interfaces of the tetramer are sequestered into the interior of the structure in an inhibited conformation. Tetramer formation is dependent on the low-complexity Mvp1 N-terminus. Remarkably, several other SNX-BARs, including some of those involved in retromer-mediated retrograde traffic from the endosome in mammalian cells, have similar but uncharacterized N-terminal extensions. This work raises the possibility that tetramerization may be a widely-conserved mechanism of regulation of membrane remodeling and ensosomal trafficking. We are currently pursuing this avenue.

   a. Sun D, Varlakhanova NV, Tornabene BA, Ramachandran R, Zhang P, **Ford MGJ**. The cryo-EM structure of the SNX-BAR Mvp1 tetramer. Nature Comm. 2020. DOI: 10.1038/s41467-020-15110-5. PMID: 32198400; PMCID: PMC7083883.

6. A second direction in our laboratory has focused on TORC1 signaling in yeast. This has resulted in a molecular characterization of a number of proteins involved in the response to nitrogen starvation. We demonstrated that the protein Pib2 is required for reactivation of TORC1 following growth arrest induced by the Tor1 inhibitor rapamycin. The EGO Complex is a key regulator of TORC1, required for reactivation of TORC1 after nitrogen starvation. We demonstrated that Pib2 is required for EGO Complex-mediated reactivation of TORC1 by the amino acids leucine and glutamine. Pib2 also regulates the distribution of TORC1 between the endosomal and vacuolar compartments. As Pib2 has a PI3P-binding FYVE domain, we proposed that Pib2 and the EGO Complex cooperate to activate TORC1 and connect phosphoinositide 3-kinase signaling and TORC1 activity.

   a. Varlakhanova NV, Tornabene BA, **Ford MGJ**. Feedback regulation of TORC1 by its downstream effectors Npr1 and Par32. Mol. Biol. Cell 2018. DOI: 10.1091/mbc.E18-03-0158. PMID: 30156471; PMCID: PMC6249832.
   b. Varlakhanova NV, Tornabene BA, **Ford MGJ**. Ivy1 is a negative regulator of Gtr-dependent TORC1 activation. J. Cell Sci. 2018. DOI: 10.1242/jcs.218305. PMID: 30097557; PMCID: PMC6140320.
   c. Varlakhanova NV, Mihalevic MJ, Bernstein KA, **Ford MGJ**. Pib2 and the EGO complex are both required for activation of TORC1. J Cell Sci. 2017. DOI: 10.1242/jcs.207910. PMID: 28993463. PMCID: PMC5702048.

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

Ongoing Research Support

5R01 GM120102 09/01/2016-06/30/2021
The Roles of the Dynamin-Related Protein Vps1 and the ESCRT Complex in Microautophagy
Role: PI
Direct costs: $197,500 p.a.

5R01 GM120102S 09/2018
Administrative Supplement
$30,658

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: Wan, William

eRA COMMONS USER NAME (credential, e.g., agency login): WANWN1

POSITION TITLE: Assistant 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
Binghamton University	B.S.	05-2008	Biochemistry
Vanderbilt University	PhD	03-2014	Chemical and Physical Biology
European Molecular Biology Laboratory	Postdoc	04-2017	Structural Biology
Max Planck Institute of Biochemistry	Postdoc	12-2019	Structural Biology

A. Personal Statement

I am currently an assistant professor in the Biochemistry department and the Center for Structural Biology at Vanderbilt University. My research program focuses on studying the structure viruses and viral machinery under near-native conditions; i.e. within intact virions or infected cells. My graduate studies in the laboratory of Gerald Stubbs at Vanderbilt University focused on the structural and biophysical characterization of the fungal prion HET-s; our work examined its mechanisms of self-assembly and cross-seeding of heterogeneous amyloid structures. My graduate training focused on structural biology and molecular biophysics, which has given me experience in a broad range of molecular biology methods, diffraction theory, and quantitative analysis. In my first postdoctoral fellowship in the laboratory of John Briggs then at the European Molecular Biology Laboratory (EMBL), I studied the structure of Ebola and Marburg viruses using intact viruses and virus-like particles. There I learned virology and gained practical experience using cryo-electron tomography (cryo-ET) and image processing. The difficulty of this project required me to aid in the development of automated data collection methods and image processing pipelines. Ultimately, our work resulted in the determination of structures of the Ebola nucleocapsid and matrix layer, providing new biological insights into the mechanisms of viral assembly that were otherwise unobtainable by studying isolated proteins. For my second postdoctoral fellowship, I joined the laboratory of Wolfgang Baumeister at the Max Planck Institute of Biochemistry. There, I broadened my experience with cryo-ET into cellular tomography, learning focused-ion beam milling (FIB-milling). I shifted my research focus to the development of image processing algorithms, culminating in an open-source software package for subtomogram averaging. Taken together, my experiences have provided me with the training and technical expertise required to assist Dr. Jackson's lab in determining the structure of SNX27/retromer coat complexes assembled on membranes.

1. William Wan, Gerald Stubbs. The fungal prion HET-s as a model for structural complexity and self-propagation in prions. *Proc. Natl. Acad. Sci. U.S.A.* (2014) 111; 5201 – 5206
2. Wim J. H. Hagen, William Wan, John A. G. Briggs. Implementation of a cryo-electron tomography tilt-scheme optimized for high resolution subtomogram averaging. J. Struct. Biol. (2017) 197; 191 – 198
3. William Wan, Larissa Kolesnikova, Mairi Clarke, Alexander Koehler, Takeshi Noda, Stephan Becker, John A.G. Briggs. Structure and assembly of the Ebola virus nucleocapsid. Nature. (2017) 551; 394 – 397

4. William Wan, Mairi Clarke, Michael Norris, Larissa Kolesnikova, Alexander Koehler, Zachary Bornholdt, Stephan Becker, Erica Ollmann Saphire, John A.G. Briggs. Ebola and Marburg virus matrix layers are locally ordered assemblies of VP40 dimers. *eLife.* (2020) 9: e59225

B. Positions and Honors

Positions and Employment

2020 – Assistant Professor, Department of Biochemistry, Vanderbilt University, Nashville TN

Honors and Awards

2008 Graduated Cum Laude, Binghamton University
2008 Dale B. Terry Memorial Award, Binghamton University
2008 Award for Excellence in Biochemistry, Binghamton University
2009-11 Molecular Biophysics Training Grant, National Institutes of Health
2010 Margaret C. Etter Student Lecturer Award, American Crystallographic Association
2011-14 National Research Service Award, National Institutes of Health
2011 Dissertation Enhancement Grant, Vanderbilt University
2012 Young Scientist Travel Grant, American Crystallographic Association
2014-16 Long-Term Fellowship, European Molecular Biology Organization

C. Contributions to Science

1. Biophysical and structural characterization of prion and amyloid assembly.

My doctoral work was focused on the structural assembly of prions: self-propagating infections proteins. The infectivity of prions generally refers to the ability of a protein to take on a prion fold that then converts its normally folded isoform into the prion fold, thus resulting in self-propagation of the prion fold. Prions generally form amyloids: filamentous protein aggregates defined by a basic cross-beta structure. Prions are most known for their roles in diseases such as bovine spongiform encephalopathy, the so-called “mad-cow disease”, while amyloids are most known for their role in neurodegenerative diseases including Alzheimer’s and Parkinson’s diseases. However, some prions are functional, where the host organism makes use of the self-propagating protein folds.

My doctoral work mainly focused on HET-s, a functional fungal prion. We performed a number of studies including characterizing the effect of types of amino-acid interactions on structure and propagation kinetics, non-biological alternate amyloid isoforms, and cross-seeding of heterogeneous amyloid structures. We proposed a mechanism for self-propagation that used relatively complex folds; this was in contrast to the simple 4-7 amino acid model systems that were dominant in the literature at the time. Since finishing my doctoral work, a large number of full-sized amyloid structures have been determined, each with relatively complex folds using amino-acid interactions similar to those we had previously characterized.

a) William Wan, Holger Wille, Jan Stöhr, Ulrich Baxa, Stanley B. Prusiner, Gerald Stubbs. Degradation of Fungal Prion HET-s(218-289) Induces Formation of a Generic Amyloid Fold. *Biophys. J.* (2012) 102; 2339 – 2344.
b) William Wan, Wen Bian, Michele McDonald, Aleksandra Kijac, David E. Wemmer, Gerald Stubbs. Heterogeneous seeding of a prion structure by a generic amyloid form of the fungal prion-forming domain HET-s(218-289). *J. Biol. Chem.* (2013) 288; 29604 – 29612.
c) William Wan, Gerald Stubbs. The fungal prion HET-s as a model for structural complexity and self-propagation in prions. *Proc. Natl. Acad. Sci. U.S.A.* (2014) 111; 5201 – 5206

2. Structure determination of Ebola and Marburg virus components within intact virions and virus-like particles.

The main focus of my first postdoctoral fellowship was to determine the structure of Ebola and Marburg viruses within intact virions and virus-like particles (VLPs). Ebola and Marburg viruses are part of the filovirus family, which take their name from their filamentous morphology. Filoviruses are assembled from a number of substructures: the nucleocapsid, the matrix layer, the membrane bilayer, and the

glycoprotein layer. The nucleocapsid contains the single-stranded RNA genome, which is encapsidated by the nucleoprotein and other viral proteins. The matrix layer binds the inner surface of the membrane bilayer and induces curvature to bud viral particles. The membrane-bilayer is taken from host cells during viral budding. The glycoprotein layer is bound to the outer membrane surface and is used for uptake into host cells. While a number of crystal structures existed for most of the viral proteins, none of them provided any insights into intermolecular interactions used in viral assembly.

Using cryo-ET and subtomogram averaging, we determined the structures of the nucleocapsid and the matrix layers within intact viruses and VLPs. Owing to the high biosafety level of filoviruses (BSL-4), these could only be studied after chemical fixation, which often disrupted samples and limited obtainable resolution. Therefore, we also used low biosafety level VLPs generated by recombinant protein expression; results from VLPs were compared with virus results to confirm biological relevance. Our structure of nucleocapsid elucidated the RNA binding interfaces and protein-protein interactions required for nucleocapsid assembly; these results contradicted a number of widespread assumptions about the proteins that play a structural role in nucleocapsids while also clarifying a number of biochemical observations on proteins used during viral infection. Our structures of matrix layers directly showed the assembly interfaces, contradicting the prevalent model in the field, which was inferred from crystallographic isoforms. Overall, our results demonstrated the importance of doing structural biology of intact systems, as these were the only systems where essential molecular interactions were directly observable.

a) William Wan, Larissa Kolesnikova, Mairi Clarke, Alexander Koehler, Takeshi Noda, Stephan Becker, John A.G. Briggs. Structure and assembly of the Ebola virus nucleocapsid. *Nature*. (2017) 551; 394 – 397
b) William Wan, Mairi Clarke, Michael Norris, Larissa Kolesnikova, Alexander Koehler, Zachary Bornholdt, Stephan Becker, Erica Ollmann Saphire, John A.G. Briggs. Ebola and Marburg virus matrix layers are locally ordered assemblies of VP40 dimers. *eLife.* (2020) 9: e59225

3. **The development of data collection and image processing methods for cryo-ET.**
The biological results listed in contribution 2 would not have been possible without substantial developments in cryo-ET methods. During my first postdoctoral fellowship, we developed a number of methods in order to meet our biological aims. Some of these methods were done in collaboration, including automated high-resolution data collection with Wim Hagen, and improved contrast-transfer correction with Beata Turoňová. Other methods such as improved processing pipelines were implemented by me and were subsequently used in a number of other biological projects with in the unit at EMBL. My second postdoctoral fellowship was primarily focused on computational methods development, resulting in a new open-source subtomogram averaging package: STOPGAP. STOPGAP bridges the needs of the high-resolution work of my first postdoc with the lower-resolution but more complex cellular tomography of my second postdoc. While STOPGAP is still in development, it is openly available on GitHub and structures determined using it have already been published.

a) Wim J. H. Hagen, William Wan, John A. G. Briggs. Implementation of a cryo-electron tomography tilt-scheme optimized for high resolution subtomogram averaging. J. Struct. Biol. (2017) 197; 191 – 198
b) Beata Turoňová, Florian K.M. Schur, William Wan, John A.G. Briggs. Efficient 3D-CTF correction for cryo-electron tomography using NovaCTF improves subtomogram averaging resolution to 3.4 Å. J. Struct. Biol. (2017) 199; 187 – 195
c) William Wan, Larissa Kolesnikova, Mairi Clarke, Alexander Koehler, Takeshi Noda, Stephan Becker, John A.G. Briggs. Structure and assembly of the Ebola virus nucleocapsid. Nature. (2017) 551; 394 – 397
d) Anna Rast, Miroslava Schaffer, Sahradha Albert, William Wan, Stefan Pfeffer, Florian Beck, Jürgen M. Plitzko, Jörg Nickelsen & Benjamin D. Engel. Biogenic regions of cyanobacterial thylakoids form contact sites with the plasma membrane. *Nature Plants.* (2019) 5; 436 – 446

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1he1ctsNY1lkw/bibliography/public/

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

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: Graham, Todd R.

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

POSITION TITLE: Professor of Biological Sciences

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
Maryville College, St. Louis, MO	B.A.	05/1984	Cell& Molecular Biology
St. Louis University, St. Louis, MO	Ph.D.	12/1988	Cell& Molecular Biology
St. Louis University, St. Louis, MO	Postdoc	8/1989	
Caltech, Pasadena, CA	Postdoc	10/1991	
UCSD, San Diego, CA	Postdoc	10/1992	

A. Personal Statement: I have been investigating molecular mechanisms underlying membrane biogenesis and protein trafficking using the budding yeast system for 32 years. We use genetic approaches to discover novel factors crucial for membrane biogenesis, then apply cell biological and biochemical approaches to define the function of these factors. We collaborate extensively with Dr. Lauren Jackson and Dr. Jason MacGurn at Vanderbilt University on our protein trafficking studies. My NIGMS-funded projects have resulted in numerous seminal discoveries including: 1) The first demonstration that type IV P-type ATPases (P4-ATPases) play critical roles in vesicle-mediated protein transport in the secretory and endocytic pathways. 2) The first unambiguous evidence that P4-ATPases are flippases that directly transport specific lipids across membranes. 3) Paradigm-establishing insight into how flippases recognize and transport their substrate. 4) Discovery of novel modes of P4-ATPase regulation and linkage to vesicle budding events. 5) Discovery that COPI binds K63-linked polyubiquitin chains and uses this interaction to recycle an exocytic SNARE from the endocytic pathway back to the Golgi. The P4-ATPases are part of an integrated series of membrane remodeling events essential for the function of the secretory and endocytic pathways, and establish phospholipid asymmetry of the plasma membrane. The function of nutrient transporters, ion channels and signaling receptors are all influenced by the asymmetric organization of lipids in the membrane. Not surprisingly, the 14 human P4-ATPases are linked a number of severe diseases, including intrahepatic cholestasis, metabolic disorder and severe neurological disease. Another line of research, funded by a Pilot and Feasibility subaward from NIDDK, is to probe the role of murine P4-ATPases (ATP10A and ATP10D) in diet induced obesity and type 2 diabetes.

I am also passionate about training the next generation of scholars and actively participate in undergraduate and graduate education where I place a strong emphasis on enhancing diversity and inclusion in STEM disciplines. Half of my current research group are underrepresented minority scholars and I am actively participating in a variety of initiatives to improve retention of minorities in the sciences. I have also had the privilege of serving the profession in many different capacities including: 1) Serving has a regular member of the MBPP NIH study section (2009-14) and NSF Cellular Organization (1997-98). I have also served as an ad hoc reviewer for multiple NSF and NIH panels (MBPP, CSF, CDF-4) and most recently for ESI-MIRA applications through ZRG1 CB-T (2020). 2) Serving on the editorial board of three different journals (*JBC*, *Frontiers* and *Cell Logistics*). 3) Peer reviewer for many different journals 4) Organizing regional (Southeast Regional Yeast Meeting) and international meetings, most recently as the Chair of the 2019 Molecular and Cellular Biology of Lipids Gordon Research Conference.

B. Positions and Honors

Positions and Employment

1992-1999 Assistant Professor of Biological Sciences, Vanderbilt University, Nashville, TN.
1999-2006 Associate Professor of Biological Sciences, Vanderbilt University, Nashville, TN.
2006- Professor of Biological Sciences, Vanderbilt University, Nashville, TN.
2007- Professor of Cell and Developmental Biology (secondary), Vanderbilt University
2018 Stevenson Professor of Biological Sciences

Other Experience and Professional Memberships

2000 - American Society for Biochemistry and Molecular Biology
1997 – 1998 NSF Cellular Organization Advisory Panel Member for the Cell Biology Program
2003 – 2010 National Institutes of Health, ad hoc reviewer for CDF-4, CSF, ZRG1, MMBP
2010 – 2014 National Institutes of Health, MBPP study section regular member
2015 - 2021 National Institutes of Health, ad hoc reviewer for MMBP, ZRG1 CB-T,
2011 - 2017 Editorial board, Cellular Logistics
2014 - Editorial board, Frontiers in Cell and Developmental Biology
2015 - Editorial board, Journal of Biological Chemistry

Honors

1984 Award for Excellence in Mathematics/Science, Maryville College, St. Louis, MO
1984 Honors graduate, Maryville College, St. Louis, MO
1988 Phi Beta Kappa, St. Louis University
1989 - 1991 American Cancer Society postdoctoral fellowship, California Institute of Technology
1996 - 2000 National Science Foundation CAREER award, MCB-9600835, Vanderbilt University
2004 Interdisciplinary Graduate Program "Teacher of the Year"
2009 Vanderbilt University College of Arts and Science "Excellence in Graduate Mentoring" award
2010 Vanderbilt University "Chancellor's Award for Research"
2014 Department of Biological Sciences Inaugural "Ellen Fanning Mentor" award
2017-19 Elected vice-chair of 2017 and chair of 2019 GRC on Molecular and Cellular Biology of Lipids
2018 Named Stevenson Chair of Biological Sciences

C. Contributions to Science (Graham lab authors in publications from last 5 years bolded)

1) Discovery that P4-ATPases play an essential role in vesicle-mediate protein transport. My early work as a postdoctoral fellow in Scott Emr's lab demonstrated a role for Sec18/NSF is all vesicular transport steps in the secretory pathway and the influence of brefeldin A on protein trafficking steps in the yeast system. As an independent investigator, I further characterized the influence of Arf mutations on Golgi membranes and initiated a screen for mutations synthetically lethal with Arf (*swa* mutants)(1a). This was the first forward genetic screen in yeast that recovered mutations in clathrin and led to the first demonstration that auxilin (Swa2) is essential for clathrin function in living cells. The *swa* mutant screen also linked a P4-ATPase to Arf-dependent vesicle budding for the first time (2a). Virtually nothing was known about this large P-type ATPase subgroup in any organism when we discovered a crucial role of the P4-ATPase Drs2 in protein transport from the Golgi. We went on to define the localization and cellular function for all five P4-ATPases in yeast, providing the first analysis of an entire repertoire of these pumps within a species (2b). We linked the function of P4-ATPases to specific vesicle budding events from the Golgi and endosomes and provided evidence Drs2 facilitates budding by increasing membrane curvature and cytosolic leaflet negative charge (2b-d). This is a unique contribution to the vesicular transport field, which has been confirmed in dozens of publications from multiple laboratories and systems, including human cells.

a. Chen, C.-Y., and T. R. Graham. 1998. An *arf1*Δ synthetic lethal screen identifies a new clathrin heavy chain conditional allele that perturbs vacuolar protein transport. *Genetics* 150: 577 – 589 PMCID: PMC1460353

b. Chen, C.-Y., M.F. Ingram, P. Rosal, and T.R. Graham (1999) Role for Drs2p, a P-type ATPase and potential aminophospholipid translocase, in yeast late Golgi function. *J. Cell Biol.* 147:1223-1236. PMCID: PMC2168089

c. Hua, Z., P. Fatheddin and T.R. Graham (2002) An essential subfamily of Drs2p-related P-type ATPases is required for protein trafficking between the Golgi complex and endosomal/vacuolar system. *Mol. Biol. Cell* **13**: 3162-3177 PMCID:PMC124150

d. Xu P, R.D. Baldridge, R.J. Chi, C.G. Burd, T.R. Graham. (2013) Phosphatidylserine flipping enhances membrane curvature and negative charge required for vesicular transport. *J Cell Biol.* 2013;202(6):875-86. PMCID:PMC3776346

2) Demonstration of P4-ATPase flippase activity along with regulatory and transport mechanisms. The P4-ATPases had been suggested to be phospholipid flippases through groundbreaking work in the Devaux, Schlegel and Williamson labs. However, this became quite controversial as conflicting reports emerged on the role of these P4-ATPases in transporting lipids and establishing membrane asymmetry. Thus, the P4-ATPases were called "putative" flippases for more than a decade. Our work provided the first conclusive biochemical evidence that P4-ATPases are indeed phospholipid flippases (2a), and defined key regulatory mechanisms for Drs2 (2b). Phosphatidylinositol 4-phosphate (PI4P) was known to have a crucial function in protein transport from the Golgi, but very few effectors of PI4P were known at the time. We found that a critical function of PI4P is to activate the flippase activity of Drs2 (2b). We went on to show that the C-terminal tail of Drs2 is an autoinhibitory domain and PI4P/ArfGEF binding to this domain stimulates flippase activity. We also provided the first mechanistic insight into how P4-ATPases recognize and flip specific phospholipid substrates. At the time, high-resolution structures of the Na^+/K^+-ATPase and Ca^{++}-ATPase were providing exquisite detail into how P-type ATPases recognize substrate cations. The canonical substrate-binding site in the center of the membrane domain seemed much too small to accommodate a phospholipid (the "giant substrate problem"). However, our data indicate that P4-ATPases are not using the canonical cation-binding pocket in the middle of the membrane domain. Rather, substrate is selected sequentially at two gates at the protein/lipid interface (2c,d).

a. Zhou, X, and T. R. Graham (2009) Reconstitution of phospholipid translocase activity with purified Drs2p, a type IV P-type ATPase from budding yeast. *Proc. Natl. Acad. Sci. USA* 106:16586-91 PMCID:PMC2757829

b. Natarajan, P, K. Liu, D. V. Patil, V.A. Sciorra, C.L. Jackson and T.R. Graham (2009) Regulation of a Golgi flippase by phosphoinositides and an ArfGEF. *Nature Cell Biol*. 11: 1421-1426 PMCID: PMC2787759

c. Baldridge, R.D. and T.R. Graham. (2012) Identification of residues defining phospholipid flippase substrate specificity of type IV P-type ATPases. *Proc. Natl. Acad. Sci. USA*. 109(6):E290-8 PMCID: PMC3277569

d. Baldridge, R.D. and T.R. Graham (2013) Two gate mechanism for phospholipid selection and transport by P4-ATPases. *Proc Natl Acad Sci USA,* 110:E358-67 PMCID: PMC3562821

3) Evidence that Neo1 is a flippase and discovery of Any1, a negative regulator of Neo1. The divergent P4B-ATPases (Neo1/TAT-5/ATP9A/b), are the most poorly understood members of this protein family. Neo1 is normally an essential gene and the lack of a β-subunit has led others to suggest the P4B-ATPases may not be flippases. Our attempts to reconstitute lipid transport activity with pure Neo1 were not successful so we sought genetic support for this proposed activity. Consistent with the hypothesis, we found that partial inactivation of Neo1 *in vivo* causes a loss of PS and PE asymmetry, suggesting these lipids are Neo1 substrates (**3a**). In addition, entry gate residues important for substrate selection in other P4-ATPases were also critical for Neo1 function in establishing PS/PE asymmetry (**3b**). Importantly, we identified a gain of function mutation in the Neo1 entry gate that enhances its PS recognition and allows Neo1-Y222S to functionally replace Drs2 in the cell (**3b**). These studies strongly imply that Neo1 is a PE/PS flippase. In collaboration with the Boone laboratory, we found that mutations in a novel, highly conserved gene called *ANY1* can bypass the essential function of Neo1 (**3c**). We suggested that Any1 could be a phospholipid scramblase that antagonizes Neo1's flippase activity (**3c**). However, we could not detect a scramblase activity with purified Any1 and genetic experiments failed to support this hypothesis. Any1 somehow segregates the function of Neo1 and Drs2 such that these flippases become redundant for cell viability in *any1*Δ cells (**3d**).

a. Takar, M., Y. Wu and T. R. Graham (2016) The essential Neo1 from budding yeast plays a role in establishing aminophospholipid asymmetry of the plasma membrane. *J. Biol. Chem.* 291:15727-39 PMCID: PMC4957055 Support: NIGMS R01GM107978

b. Huang, Y., M. Takar, T. R. Graham (2020) Conserved mechanism of phospholipid substrate recognition by the P4-ATPase Neo1 from Saccharomyces cerevisiae. *BBA-Mol Cell Biol Lipids* 1865(2):158581 PMCID:PMC6957724. Support: NIGMS R01GM107978

c. van Leeuwen, J., C. Pons, J.C. Mellor, T.N. Yamaguchi, H. Friesen, J. Koschwanez, M.M. Usaj, M. Pechlaner, **M. Takar**, 29 authors, **T.R. Graham**, C.L. Myers, B.J. Andrews, F.P. Roth, and C. Boone. **(2016)** Exploring genetic suppression interactions on a global scale. *Science* 354: 599 PMCID:PMC 5562937. Support: NIGMS R01GM107978

d. Takar, M., Y. Huang and T.R. Graham (2019) The PQ-loop protein Any1 segregrates Drs2 and Neo1 functions required for viability and plasma membrane asymmetry. *J. Lipid Res.* 60:1032-42 PMCID: PMC6495175. Support: NIGMS R01GM107978

4) Directed evolution of new substrate specificity and discovery of GlcCer flippases. For the P4-ATPase structure/function studies, we had created a yeast library of ~5000 *DNF1* alleles harboring 1 – 3 mutations on average and targeted by error-prone PCR to M1-6 (2d). We previously screen this library for variants that gain the ability to flip PS or diminish PC transport activity. To determine how P4-ATPases distinguish glycerophospholipid from sphingolipid, we used a flow-cytometry based screen to identify gain-of-function (neomorphic) variants that flip sphingomyelin, which identified a key exit gate residue and a role for M6 (**4a**). While exploring the substrate specificity of these variants, we 1) surprisingly discovered that ***wild-type*** Dnf1 and Dnf2 robustly transports glucosylceramide (GlcCer); 2) identified ATP10A and ATP10D as human GlcCer flippases; and 3) mapped residues critical for GlcCer selection (**4b**). This study provided a novel link between P4-ATPase function, sphingolipid metabolism and metabolic disorder. To further address mechanism, we found the very different lipids GlcCer and PC mutually compete for transport, suggesting a common translocation path, and that substrate specificity of the pumps is strongly modulated by the lipid composition of the membrane (**4c**). In collaboration with the Li lab, we now have the cryo-EM structure of Dnf1/2-Lem3, which is providing critical new mechanistic insight into how substrate lipid is recognized at the exit gate (**4d**)(further described in proposal).

a. Roland, B.P. and T. R. Graham (2016) Directed evolution of a sphingomyelin flippase reveals mechanisms of substrate backbone discrimination by a P4-ATPase. *Proc Natl Acad Sci USA* 113:E4460-6. PMCID:PMC4978280. Support: NIGMS R01GM107978

b. Roland, B.P., T. Naito, J.T. Best, C. Arnaiz-Yepez, H. Takatsu, R.Y. Yu, H.-W. Shin, T.R. Graham (2019) Yeast and human P4-ATPases transport glycosphingolipids using conserved structural motifs. *J. Biol. Chem*. jbc.RA118.005876. PMCID:PMC6369285. Support: NIGMS R01GM107978

c. Jain BK, Roland BP, Graham TR. (2020) Exofacial membrane composition and lipid metabolism regulates plasma membrane P4-ATPase substrate specificity. *J Biol Chem*. 2020 295(52):17997-18009. PubMed PMID: 33060204. PMCID: In progress. Support: NIGMS R01GM107978

d. Bai*, L., Q. You***, B.K. Jain*,** H.D. Duan, A. Kovach**, T.R. Graham,** H. Li **(2020)** Transport mechanism of P4 ATPase phosphatidylcholine flippases, *Elife*, 9 PMCID: PMC7773333 Support: NIGMS R01GM107978

5) Defining the importance of specific P4-ATPase substrates in vesicle trafficking and Snc1 recycling pathways. Through the structure/function studies, we have learned how to mutationally tune the substrate specificity of P4-ATPases. We can disrupt PS flip by Drs2 without perturbing its ability to flip PE. We also created gain-of-function mutations in Dnf1 that allow it to flip PS or sphingomyelin without altering its ability to transport PC and PE (2d,**4b**). With these "designer flippases", we could show that translocation of PS is absolutely required for the budding of AP-1 clathrin-coated vesicles from the TGN, the budding of vesicles from early endosomes to recycle Snc1 to the Golgi (1d), and sorting of "raft"-associated proteins into exocytic vesicles (**5a**). The Snc1 recycling pathway was poorly understood and so we further characterized this route. Surprisingly, we found this Drs2-dependent transport step requires Snc1 K63-linked polyubiquitination and recognition of this sorting signal by COPI (**5b**). Sorting nexins (Snx4/41) were also implicated in Snc1 recycling but we found that these proteins and retromer mediate distinct and parallel endosome to Golgi pathways with Drs2/COPI (**5c**). While P4-ATPase lipid transport is required for vesicle budding from the Golgi and endosomes, we also provided the first *in vivo* evidence that PE asymmetry produced by Neo1 is required for SNARE-mediated homotypic fusion of vacuoles (**5d**)

a. Hankins, H., Y.Y Sere, N.S. Diab, A.K. Menon and T.R. Graham (2015) Phosphatidylserine translocation at the yeast trans-Golgi network regulates protein sorting into exocytic vesicles. Mol Biol Cell 26:4674-85 PMCID:PMC4678023. Support: NIGMS R01GM107978

b. Xu, P., H.M. Hankins, C. MacDonald, **S.J. Erlinger,** M.N. Frazier, **N.S. Diab,** R.C. Piper, L.P. Jackson, J.A. MacGurn**, and T.R. Graham (2017)** COPI mediates recycling of an exocytic SNARE by recognition of a ubiquitin sorting signal. Elife 6:e28342 PMCID: PMC5663479. Support: R01GM118452

c. Best, J.T., P. Xu, J.G McGuire, S.N. Leahy and T.R. Graham (2020) Yeast synaptobrevin, Snc1, engages distinct routes of postendocytic recycling mediated by a sorting nexin, Rcy1-COPI and retromer. Mol. Biol. Cell 31(9):944-62 PMCID: PMC7185969. Support: R01GM118452

d. Wu, Y., M. Takar, A.A. Cuentas-Condori and T. R. Graham (2016) Neo1 and phosphatidylethanolamine contribute to vacuole membrane fusion in *Saccharomyces cerevisiae.* Cell. Logistics 6:e1228791 PMCID:PMC5058351. NIGMS R01GM107978

Complete List of Published Work in MyBibliography
http://www.ncbi.nlm.nih.gov/sites/myncbi/todd.graham.1/bibliography/40661031/public/?sort=date&direction=ascending.

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

1 R01 GM107978-05 Graham (PI) 02/01/18 – 01/31/22
NIGMS "P4-ATPase mechanism of phospholipid translocation"
This study is to define how P4-ATPases recognize their phospholipid substrate and flip them across the bilayer to establish membrane asymmetry.
The focus of this proposal is to perform structure/function analyses of Drs2 and Dnf1 to define the phospholipid translocation pathway and to define the substrates of Neo1 and mammalian P4-ATPases.

5 R01GM118452-04 Graham (PI) 05/01/16 - 12/31/2021
NIGMS "Mechanisms of protein transport between Golgi and endosomes"
The focus of this proposal is to study the mechanism of bidirectional transport between the Golgi and endosomes with a focus on COPI in the trafficking of ubiquitinated cargos.

2 P30 DK020593 (Powers, PI; subaward PI,Graham)) 04/01/19 – 03/31/2021
NIDDK Vanderbilt Diabetes Research and Training Center Pilot and Feasibility Award
"Influence of *Atp10a* and *Atp10d* on diet induced obesity and insulin resistance"
The proposed studies on *Atp10a/d* in mice provide a unique approach to an understudied and potentially highly significant contributor to insulin resistance and human disease.

1 T32 GM137793 (Patton, PI/PD; Friedman, MPI; and Graham, MPI) 7/1/21-6/30/26
NIGMS. "Cellular, Molecular, and Biochemical Sciences Training Program"
Training grant that provides unique educational and training experience for a diverse group of graduate students engaging in biomedical research at Vanderbilt University.

Research support completed in the past three years

1 R13 DK121357-01 Graham (PI) 2019
NIDDK, "Molecular and Cellular Biology of Lipids GRC". (For support of a Gordon Research Conference)

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: Kendall, Amy

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

POSITION TITLE: Laboratory Manager

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
Vanderbilt University, Nashville, TN	BS	05/1993	Elementary Education / Natural Sciences
Vanderbilt University, Nashville, TN	MS	05/2000	Biological Sciences

A. Personal Statement

Beginning with my graduate work in the mid-nineties, I have always worked at the intersection of structural methods that have included x-ray fiber diffraction, xray crystallography, and negative stain and cryo-electron microscopy. My studies of flexible filamentous plant viruses eventually led to studies of disordered amyloid and prion filaments, and this work has prepared me well for structural studies of malleable membrane trafficking proteins including retromer. As a senior staff scientist, I have helped to drive the adoption of negative stain and cryoEM methods by our laboratory, and have served as the EM "point person" for more than 20 years. I have trained students in microscopy methods and written protocols for sample preparation, instrument use, image processing and validation, and publication production. I have attended workshops and conferences in order to remain up-to-date on current topics in microscopy, and have disseminated this information to our laboratory and others at Vanderbilt.

1. Xie B, Jung C, Chandra M, Engel A, Kendall AK, Jackson LP. The Glo3 GAP crystal structure supports the molecular niche model for ArfGAPs in COPI coats. Adv Biol Regul. 2021 Jan;79:100781. PubMed Central PMCID: PMC7920988.
2. Kendall AK, Xie B, Xu P, Wang J, Burcham R, Frazier MN, Binshtein E, Wei H, Graham TR, Nakagawa T, Jackson LP. Mammalian Retromer Is an Adaptable Scaffold for Cargo Sorting from Endosomes. Structure. 2020 Apr 7;28(4):393-405.e4. PubMed Central PMCID: PMC7145723.
3. Tuttle MD, Comellas G, Nieuwkoop AJ, Covell DJ, Berthold DA, Kloepper KD, Courtney JM, Kim JK, Barclay AM, Kendall A, Wan W, Stubbs G, Schwieters CD, Lee VM, George JM, Rienstra CM. Solid-state NMR structure of a pathogenic fibril of full-length human α-synuclein. Nat Struct Mol Biol. 2016 May;23(5):409-15. PubMed Central PMCID: PMC5034296.
4. Kendall A, McDonald M, Bian W, Bowles T, Baumgarten SC, Shi J, Stewart PL, Bullitt E, Gore D, Irving TC, Havens WM, Ghabrial SA, Wall JS, Stubbs G. Structure of flexible filamentous plant viruses. J Virol. 2008 Oct;82(19):9546-54. PubMed Central PMCID: PMC2546986.

B. Positions and Honors

Positions and Employment

2005 - Laboratory Manager, Vanderbilt University, Nashville, TN
2002 - 2005 Research Assistant III, Vanderbilt University, Nashville, TN
1999 - 2002 Research Assistant II, Vanderbilt University, Nashville , TN

C. Contribution to Science

1. Structural studies of membrane trafficking proteins

   The diverse pathways of membrane trafficking are essential to maintaining cellular homeostasis and function. We have used a combination of biochemical and structural methods to determine details of the interaction between AP4 and its accessory protein tepsin, and have explored the range of conformations adopted by the endosomal trafficking protein retromer by cryoEM and disrupted its function in yeast. Continued study of these and other trafficking proteins will allow us to characterize the mechanisms by which transport is executed within the cell and to describe the associated proteins that make this transport possible.

   a. Xie B, Jung C, Chandra M, Engel A, Kendall AK, Jackson LP. The Glo3 GAP crystal structure supports the molecular niche model for ArfGAPs in COPI coats. Adv Biol Regul. 2021 Jan;79:100781. PubMed Central PMCID: PMC7920988.
   b. Kendall AK, Xie B, Xu P, Wang J, Burcham R, Frazier MN, Binshtein E, Wei H, Graham TR, Nakagawa T, Jackson LP. Mammalian Retromer Is an Adaptable Scaffold for Cargo Sorting from Endosomes. Structure. 2020 Apr 7;28(4):393-405.e4. PubMed Central PMCID: PMC7145723.
   c. Archuleta TL, Frazier MN, Monken AE, Kendall AK, Harp J, McCoy AJ, Creanza N, Jackson LP. Structure and evolution of ENTH and VHS/ENTH-like domains in tepsin. Traffic. 2017 Sep;18(9):590-603. PubMed Central PMCID: PMC5567745.
   d. Frazier MN, Davies AK, Voehler M, Kendall AK, Borner GH, Chazin WJ, Robinson MS, Jackson LP. Molecular Basis for the Interaction Between AP4 β4 and its Accessory Protein, Tepsin. Traffic. 2016 Apr;17(4):400-15. PubMed Central PMCID: PMC4805503.

2. Structural studies of amyloid and prion proteins.

   Structural studies of self-propagating amyloids (prions) are essential to understand the mechanism of prion self-propagation and molecular toxicity, and structural studies of both prion and non-prion amyloids are required for the rational design of drugs to treat these diseases. We used x-ray fiber diffraction in combination with solid state NMR and negative stain electron microscopy to determine low resolution structures of Aβ, the amyloid implicated in Alzheimer's disease, and α-synuclein, the amyloid implicated in Parkinson's disease. We also used x-ray fiber diffraction to compare a number of different types of natural and synthetic PrP prions.

   a. Barran-Berdon AL, Ocampo S, Haider M, Morales-Aparicio J, Ottenberg G, Kendall A, Yarmola E, Mishra S, Long JR, Hagen SJ, Stubbs G, Brady LJ. Enhanced purification coupled with biophysical analyses shows cross-β structure as a core building block for Streptococcus mutans functional amyloids. Sci Rep. 2020 Mar 20;10(1):5138. PubMed Central PMCID: PMC7083922.
   b. Tuttle MD, Comellas G, Nieuwkoop AJ, Covell DJ, Berthold DA, Kloepper KD, Courtney JM, Kim JK, Barclay AM, Kendall A, Wan W, Stubbs G, Schwieters CD, Lee VM, George JM, Rienstra CM. Solid-state NMR structure of a pathogenic fibril of full-length human α-synuclein. Nat Struct Mol Biol. 2016 May;23(5):409-15. PubMed Central PMCID: PMC5034296.
   c. Wan W, Wille H, Stöhr J, Kendall A, Bian W, McDonald M, Tiggelaar S, Watts JC, Prusiner SB, Stubbs G. Structural studies of truncated forms of the prion protein PrP. Biophys J. 2015 Mar 24;108(6):1548-1554. PubMed Central PMCID: PMC4375555.
   d. Wille H, Bian W, McDonald M, Kendall A, Colby DW, Bloch L, Ollesch J, Borovinskiy AL, Cohen FE, Prusiner SB, Stubbs G. Natural and synthetic prion structure from X-ray fiber diffraction. Proc Natl Acad Sci U S A. 2009 Oct 6;106(40):16990-5. PubMed Central PMCID: PMC2761340.

3. Structural studies of filamentous plant viruses.

   Filamentous plant viruses have long been used as models for viral structure and assembly. Using a combination of x-ray fiber diffraction and cryo-electron microscopy, we determined the low resolution structures of a number of different flexible filamentous plant viruses including potato virus X and soybean mosaic virus, and the low resolution structure of a rigid filamentous plant virus, barley stripe mosaic virus. These low resolution structures allowed us to speculate about the relationships between the filamentous plant viruses and provided information that might be used to design modified coat proteins for peptide expression and conferral of resistance on host plants.

   a. Stubbs G, Kendall A. Fibre diffraction in the analysis of filamentous virus structure. Crystallography Reviews. 2016; 22:84-101. DOI: 10.1080/0889311X.2015.1025771
   b. Kendall A, Williams D, Bian W, Stewart PL, Stubbs G. Barley stripe mosaic virus: structure and relationship to the tobamoviruses. Virology. 2013 Sep 1;443(2):265-70. PubMed PMID: 23725818.
   c. Kendall A, McDonald M, Bian W, Bowles T, Baumgarten SC, Shi J, Stewart PL, Bullitt E, Gore D, Irving TC, Havens WM, Ghabrial SA, Wall JS, Stubbs G. Structure of flexible filamentous plant viruses. J Virol. 2008 Oct;82(19):9546-54. PubMed Central PMCID: PMC2546986.

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: Mintu Chandra

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

POSITION TITLE: Post Doctoral Research Scholar

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
Jadavpur University, Kolkata, India	M.Sc.	06/2010	Life Science and Biotechnology
Indian Institute of Science Education and Research (IISER) Bhopal, Madhya Pradesh, India	Ph.D.	02/2016	Structural Biology
Institute for Molecular Biosciences (IMB), University of Queensland, St. Lucia, Australia	Postdoctoral	12/2018	Structural Biology
Department of Biological Sciences & Biochemistry, Vanderbilt University, Nashville, TN, USA	Postdoctoral	Ongoing	Structural Biology

A. Personal Statement

My long-standing interest has been in the protein-protein and protein-small molecule interactions with the help of protein biochemistry, biophysics, X-ray crystallography and Cryo-EM. We all have evolved highly sophisticated protein machineries to control the flow of trans-membrane molecules and lipids between different organelles. Any disruption of these processes are linked to numerous diseases including neurodegenerative disorders and cancer. Using cutting-edge structural, molecular and cellular approaches I aim to determine how these trafficking machineries are assembled and regulated at the molecular level. My future goal is to uncover the details of the architecture of the functional membrane-associated complex, retromer-SNXs (SNX27 and SNX-BAR), assembled in the presence of PI3P-enriched nanodiscs. This will provide a molecular foundation for identifying target sites and rational design for developing potential therapeutics.

- *Xuan Ling Hilary Yong, Lingrui Zhang, Liming Yang, Xiumin Chen, Jing Zhi Anson Tan, Xiaojun Yu,* ***Mintu Chandra****, Emma Livingstone, Jocelyn Widagdo, Marta M. Vieira, Katherine W. Roche, Joseph W. Lynch, Angelo Keramidas, Brett M. Collins, Victor Anggono. Regulation of NMDA receptor trafficking and gating by activity-dependent CaMKIIα phosphorylation of the GluN2A subunit.* ***Cell Reports.*** ***(Accepted article - D-21-00643R1).***

- ***Chandra M***[#]*, Kendall A, Jackson L.P*[#]*. Towards understanding the molecular role of SNX27/retromer in human health and disease.* ***Front. Cell Dev. Biol****. 2021 March 22; doi: 10.3389/fcell.2021.642378 (*[#]***Corresponding authors).***

- *Xie B, Jung C,* ***Chandra M****, Engel A, Kendall A, Jackson L.P. The Glo3 GAP crystal structure supports the molecular niche model for ArfGAPs in COPI coats.* ***Advances in Biological Regulation****. 2021 (DOI: https://doi.org/10.1016/j.jbior.2020.100781).*

- *Snider C, **Chandra M**, McDonald N, Willet A, Collier S, Ohi M, Jackson L.P, Gould K. Opposite surfaces of the Cdc15 F-BAR domain create a membrane platform that coordinates cytoskeletal and signaling components for cytokinesis. **Cell Reports.** 33, 108526, December 22, 2020. https://doi.org/10.1016/j.celrep.2020.108526.*

- ***Chandra M**[#], Kendall A, Jackson L.P[#]. Unveiling the cryo-EM structure of retromer. **Biochemical Society Transactions.** 2020 September 14; 0; 1–12. doi.org/10.1042/BST20200552 ([#]**Corresponding authors**).*

- ***Chandra M**, Chin YK, Mas C, Feathers JR, Paul B, Datta S, Chen KE, Jia X, Yang Z, Norwood SJ, Mohanty B, Bugarcic A, Teasdale RD, Henne WM, Mobli M, Collins BM. Classification of the human phox homology (PX) domains based on their phosphoinositide binding specificities. **Nat Commun.** 2019 Apr 4;10(1):1528. doi: 10.1038/s41467-019-09355-y.*

- *Ugrankar R, Bowerman J, Hariri H, **Chandra M**, Chen KE, Bossanyi MF, Datta S, Rogers S, Eckert KM, Vale G, Victoria A, Fresquez J, McDonald JG, Jean S, Collins BM, and Henne WM. Drosophila Snazarus regulates a lipid droplet sub-population at plasma membrane-droplet contacts in fat body adipocytes. **Developmental Cell**. 2019 Aug 15; doi: 10.1016/j.devcel.2019.07.021.*

- *Chen KE, Tillu VA, **Chandra M**, Collins BM. Molecular basis for membrane recruitment by the PX and C2 domains of class II phosphoinositide 3-kinase-C2α. **Structure.** 2018 Dec 4;26(12):1612-1625.e4. doi: 10.1016/j.str.2018.08.010. Epub 2018 Oct 4.*
- ***Chandra M**, Collins BM. The Phox Homology (PX) Domain. **Adv Exp Med Biol.** 2018 Mar 23. doi: 10.1007/5584_2018_185. [Epub ahead of print].*

- *Yang Z, Follett J, Kerr MC, Clairfeuille T, **Chandra M**, Collins BM, Teasdale RD. Sorting Nexin 27 (SNX27) regulates the trafficking and activity of the glutamine transporter ASCT2. **J Biol Chem**. 2018 Mar 21. pii: jbc.RA117.000735. doi: 10.1074/jbc.RA117.000735. [Epub ahead of print].*

- *Clairfeuille T, Mas C, Chan AS, Yang Z, Tello-Lafoz M, **Chandra M**, Widagdo J, Kerr MC, Paul B, Mérida I, Teasdale RD, Pavlos NJ, Anggono V, Collins BM. A molecular code for endosomal recycling of phosphorylated cargos by the SNX27–retromer complex. **Nature Structural & Molecular Biology** (2016) doi:10.1038/nsmb.3290.*

B. Employment, Honors, & Service

Employment

February, 2019 - Present	Post Doctoral Research Scholar, Dept. of Biological Sciences, Vanderbilt University, Nashville, TN, USA.
May, 2016 – December, 2018	Post Doctoral Research Officer, Institute for Molecular Biosciences (IMB), University of Queensland, St. Lucia, Australia.

Honors

2019	**William N Pearson Fellowship Award 2019** in Nutritional Science and Metabolism, Dept. of Biochemistry, Vanderbilt University, Nashville, TN, USA.
2015	**Best Poster Award** at the 13th Conference of the Asian Crystallography Association (AsCA), Science City, Kolkata, India, December 05-08th, 2015.
2015	**International Travel Grant from Department of Science and Technology (DST) -** To attend the Biophysical Society conference November 16-20th, 2015 at Spier Wine Estate, Stellenbosch, Western Cape, South Africa.

C. Contributions to Science

Publication list
https://www.ncbi.nlm.nih.gov/pubmed/?term=mintu+chandra

1. To understand the retromer coat assembly and regulation on endosomes and to provide a fundamentally improved understanding of the role of endosomal membrane recycling in cellular homeostasis.

My recent work on PX domain for their phospholipid preferences has provided insight into the molecular basis of membrane trafficking mediated by the Phox Homology (PX) domains. The systematic screening of all human PX domains for their phospholipid preferences and the PX domain structures revealed two distinct binding sites that explain their lipid specificities, providing a basis for defining and predicting the functional membrane interactions of the entire PX domain protein family. This work has recently been published in Nature Communications journal.
Currently, I am trying to understand the molecular mechanisms by which the Retromer complex (VPS26/VPS35/VPS29 subunits) interacts with SNX27. Retromer plays a fundamental role in nutrition. In metazoans, the Retromer/SNX27 complex recycles a number of critical nutrient receptors, including glucose transporter type 1 (GLUT1) and the iron transporter DmtI. Although SNX27 appears to facilitate cargo specificity of Retromer, regulatory mechanisms for SNX27/Retromer assembly during receptor recycling remain elusive. I aim to identify novel regulators associated with SNX27/Retromer assembly and investigate their role in GLUT1 recycling to the plasma membrane. Subsequently, I will pursue the structural studies of the complex on its own and assembled in the presence of PI3P-enriched nanodiscs. This work will provide critical insight into how retromer assembles in the presence of cargo. In order to get insights into the complex assembly of SNX27-retromer, I have successfully reconstituted the retromer-SNX27 complex at the highest purity level and currently obtaining initial 2D class averages.

- *Xuan Ling Hilary Yong, Lingrui Zhang, Liming Yang, Xiumin Chen, Jing Zhi Anson Tan, Xiaojun Yu,* ***Mintu Chandra****, Emma Livingstone, Jocelyn Widagdo, Marta M. Vieira, Katherine W. Roche, Joseph W. Lynch, Angelo Keramidas, Brett M. Collins, Victor Anggono. Regulation of NMDA receptor trafficking and gating by activity-dependent CaMKIIα phosphorylation of the GluN2A subunit.* ***Cell Reports.*** ***(Accepted article - D-21-00643R1).***

- ***Chandra M***[#]*, Kendall A, Jackson L.P*[#]*. Towards understanding the molecular role of SNX27/retromer in human health and disease.* ***Front. Cell Dev. Biol****. 2021 March 22; doi: 10.3389/fcell.2021.642378 (*[#]***Corresponding authors)*****.**

- ***Chandra M***[#]*, Kendall A, Jackson L.P*[#]*. Unveiling the cryo-EM structure of retromer.* ***Biochemical Society Transactions****. 2020 September 14; 0; 1–12. doi.org/10.1042/BST20200552 (*[#]***Corresponding authors)*****.**

- ***Chandra M****, Chin YK, Mas C, Feathers JR, Paul B, Datta S, Chen KE, Jia X, Yang Z, Norwood SJ, Mohanty B, Bugarcic A, Teasdale RD, Henne WM, Mobli M, Collins BM. Classification of the human phox homology (PX) domains based on their phosphoinositide binding specificities.* ***Nat Commun.*** *2019 Apr 4;10(1):1528. doi: 10.1038/s41467-019-09355-y.*

- *Ugrankar R, Bowerman J, Hariri H,* ***Chandra M****, Chen KE, Bossanyi MF, Datta S, Rogers S, Eckert KM, Vale G, Victoria A, Fresquez J, McDonald JG, Jean S, Collins BM, and Henne WM. Drosophila Snazarus regulates a lipid droplet sub-population at plasma membrane-droplet contacts in fat body adipocytes.* ***Developmental Cell****. 2019 Aug 15; doi: 10.1016/j.devcel.2019.07.021.*

- *Chen KE, Tillu VA,* ***Chandra M****, Collins BM. Molecular basis for membrane recruitment by the PX and C2 domains of class II phosphoinositide 3-kinase-C2α.* ***Structure.*** *2018 Dec 4;26(12):1612-1625.e4. doi: 10.1016/j.str.2018.08.010. Epub 2018 Oct 4.*
- ***Chandra M****, Collins BM. The Phox Homology (PX) Domain.* ***Adv Exp Med Biol.*** *2018 Mar 23. doi: 10.1007/5584_2018_185. [Epub ahead of print].*

- *Yang Z, Follett J, Kerr MC, Clairfeuille T,* ***Chandra M****, Collins BM, Teasdale RD. Sorting Nexin 27 (SNX27) regulates the trafficking and activity of the glutamine transporter ASCT2.* ***J Biol Chem****. 2018 Mar 21. pii: jbc.RA117.000735. doi: 10.1074/jbc.RA117.000735. [Epub ahead of print].*

- *Clairfeuille T, Mas C, Chan AS, Yang Z, Tello-Lafoz M, **Chandra M**, Widagdo J, Kerr MC, Paul B, Mérida I, Teasdale RD, Pavlos NJ, Anggono V, Collins BM. A molecular code for endosomal recycling of phosphorylated cargos by the SNX27–retromer complex. **Nature Structural & Molecular Biology** (2016) doi:10.1038/nsmb.3290.*

2. Biochemical and X-ray crystallographic studies on *Eh*RabX3, a novel GTPase from *Entamoeba histolytica* with tandem G-Domains

My doctoral dissertation was focused on biochemical and structural studies of *Eh*RabX3, a unique and catalytically inefficient tandem Rab GTPase from *Entamoeba histolytica*. I identified how the conventional GTP/GDP cycle is regulated in *Eh*RabX3 and what is the molecular basis of low catalytic efficiency of this atypical Rab protein. The biochemical, structural and functional investigation carried out on EhRabX3 would allow us to design potential inhibitors for the better treatment of intestinal amebiasis.

- ***Chandra M*[#]**, *Srivastava VK[#], Saito-Nakano Y, Nozaki T and Datta S. Crystal Structure analysis of wild-type and fast hydrolyzing mutant of EhRabX3, a tandem Ras superfamily GTPase from Entamoeba histolytica. **J Mol Biol**. 2015 Nov 7. pii: S0022-2836(15)00624-5. doi: 10.1016/j.jmb.2015.11.003). (# Co-first authors).*

- ***Chandra M*[#]**, *Srivastava VK[#] and Datta S. Crystallization and preliminary X-ray analysis of RabX3, a tandem GTPase from Entamoeba histolytica. **Acta Crystallographica Section F**: Structural Biology Communications, Acta Cryst. (2014). F70, 933–937. (# Co-first authors).*
- ***Chandra M**, Mukherjee M, Srivastava VK, Saito-Nakano Y, Nozaki T, Datta S. Insights into the GTP/GDP Cycle of RabX3, a Novel GTPase from Entamoeba histolytica with Tandem G-Domains. **Biochemistry**, January 28, 2014 (Article), DOI: 10.1021/bi401428f.*

- ***Chandra M**, Datta S. Role of cysteine residues in the redox-regulated oligomerization and nucleotide binding to EhRabX3. **Mol Biochem Parasitol**. 2016 Aug;208(2):84-90. doi: 10.1016/j.molbiopara.2016.06.009. Epub 2016 Jul 30.*