BIOGRAPHICAL SKETCH

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NAME: Port, Sarah Alexandra

eRA COMMONS USER NAME: SAPORT

POSITION TITLE: Postdoctoral Researcher

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Technische Universität München, Germany	BS	09/2008	Molecular Biotechnology
Technische Universität München, Germany	MS	12/2010	Molecular Biotechnology
Georg-August-Universität Göttingen, Germany	PhD	09/2015	Molecular Biology
Georg-August-Universität Göttingen, Germany	postdoc	04/2016	Molecular Biology
Princeton University, USA	postdoc	present	Structural Biology

A. Personal Statement

My long-term research interest is to understand the mechanisms underlying the specificity and efficiency of intracellular transport on a molecular level. For my graduate studies, I focused on the molecular interactions of transport complexes and the nuclear pore complex during nucleocytoplasmic transport. We collaborated closely with experts to apply cross-linking mass spectrometry and X-ray crystallography methods to a complex of nuclear transport receptor, transport cargo, RanGTPase and a fragment of the nuclear pore complex. This work culminated in the first crystal structure of a nuclear transport complex bound to a component of the nuclear pore complex, thereby showing that the phenylalanine-glycine repeats of the nucleoporin interact with hydrophobic pockets on the surface of the transport receptor.
For my postdoc, I joined the Hughson lab at Princeton University to study the structural basis of vesicle trafficking, with a focus on the molecular interactions of proteins involved in membrane tethering and fusion. My graduate research has provided a strong foundation in many of the areas of expertise needed for my project. Specifically, I have extensive experience in cloning, protein expression and purification and the analysis of protein interactions *in vitro* and *in vivo*, as well as substantial exposure to crystallographic and cryo-EM-based structural studies of macromolecular complexes. I am also expert in immunoprecipitation, Western blotting, fluorescence and confocal microscopy, and flow cytometry. In 2019, I was selected for a 3-month embedded training program at the National Center for CryoEM Access and Training (NCCAT) in New York, where I will receive intensive, personalized training in all aspects needed to solve a high-resolution protein structure by cryo-electron microscopy.

B. Positions and Honors

Positions and Employment

2015-2016 Postdoctoral Researcher, Department of Molecular Biology, Universitätsmedizin Göttingen, Germany
2016- Postdoctoral Researcher, Department of Molecular Biology, Princeton University, USA

Other Experiences

2019 National Center for CryoEM Access and Training (NCCAT), embedded training (project NCCAT-TP1-SP181214)

Professional Memberships

2016-2017 American Heart Association
2017- Gesellschaft für Biochemie und Molekularbiologie (German Society for Biochemistry and Molecular Biology)
2017- American Association for the Advancement of Science
2017- American Society for Cell Biology

Honors

2015 *Summa cum laude* (PhD thesis), Georg-August-Universität Göttingen

C. Contributions to Science

For my graduate research as well as my first year of postdoctoral research, I worked on nucleocytoplasmic transport. The sole gateways for transport between the cytoplasm and nucleus are the nuclear pore complexes (NPCs). A subset of the nucleoporins that make up the NPCs contain hydrophobic and intrinsically disordered stretches of phenylalanine-glycine (FG-) repeats, which form a permeability barrier. As a result, most molecules cannot diffuse through the NPC, but need to be actively transported by interaction with nuclear transport receptors. The major receptor for nuclear export is CRM1, which upon binding of the small GTPase Ran loaded with GTP, exports cargoes by interacting with their nuclear export signals (NES). The nucleoporin with the longest FG-repeat domain is Nup214.
For my postdoc, I study the structure and function of the multisubunit tethering complex HOPS, a 660 kDa hetero-hexameric complex involved in the tethering a fusion of late endosomes. In a first step, we investigated the functional interactions of the HOPS subunit Vps33 with SNARE proteins using single-molecule force microscopy.

1. Structural and functional characterization of the interaction of the nuclear export receptor CRM1 with the nucleoporin Nup214.

We determined the structure of a FG-repeat-containing fragment of Nup214 bound to a CRM1 export complex by X-ray crystallography. This was the first time the interaction of any transport receptor with a FG-nucleoporin had been visualized at high resolution. The interactions our structure revealed were verified using biochemical and cell-based assays and cross-linking mass spectrometry. Combining the insights in the molecular mechanisms of the interaction between nucleoporins and transport receptors with observations of the localization and mobility of two leukemogenic Nup214-fusions proteins in the cell, revealed the molecular basis of how nucleocytoplasmic transport can be disturbed in disease.

a) Port, S.A., Monecke, T., Dickmanns, A., Spillner, C., Hofele, R., Urlaub, H., Ficner, R., and Kehlenbach, R.H. (2015) Structural and Functional Characterization of CRM1-Nup214 Interactions Reveals Multiple FG-Binding Sites Involved in Nuclear Export. *Cell Reports*, 13(4):690-702, PMID: 26489467

b) Monecke T, Dickmanns A, Weiss,MS, Port SA, Kehlenbach RH, Ficner R (2015) Combining dehydration, construct optimization and improved data collection to solve the crystal structure of a CRM1-RanGTP-SPN1-Nup214 quarternary nuclear export complex. *Acta Crystallographica Section F,* 71(Pt 12):1481-1487, PMCID: PMC4666476

c) Port SA, Mendes A, Valkova C, Fahrenkrog B, Kaether C, and Kehlenbach RH (2016). The oncogenic fusion proteins SET-Nup214 and SQSTM1-Nup214 form dynamic nuclear bodies and inhibit nuclear protein- and mRNA export, *The Journal of biological chemistry*, 291(44):23068-23083, PMCID: PMC5087727

2. Development of assays to monitor CRM1-mediated nuclear export and quantify the involved protein interactions.

Our original assay recapitulating CRM1-dependent nuclear export made use of a stable cell line expressing GFP-NFAT and was therefore not widely available to other researchers. To fix this situation, we extended the assay using cells transiently transfected with various cargo proteins and a transfection marker. CRM1-, Ran- and energy-dependent nuclear export was reconstituted in digitonin-permeabilized cells and quantified by flow cytometry. This simplified assay can be applied for the in vitro analysis and characterization of any potential CRM1 cargo without the need for a stable cell line.
Furthermore, we developed a bead-based, semi-quantitative assay to analyze the interaction of Cy3-labeled CRM1 with a variety of cargoes and transport factors by flow cytometry. Compared to conventional gel-based pulldown assays, the power of the bead–based assay lies in the analysis of protein-protein interactions of very different strengths, covering a detection range of three orders of magnitude. The assay therefore allowed for the comparison of CRM1 binding to very different cargoes and nucleoporin fragments, and should be extendable to the analysis of other proteins.

a) Kehlenbach RH and Port SA (2016) Analysis of CRM1-dependent Nuclear Export in Permeabilized Cells. *Methods in Molecular Biology*, 1411:489-501, PMID 27147061

b) Thakar K, Karaca S, Port SA, Urlaub H, Kehlenbach RH (2013) Identification of CRM1-dependent Nuclear Export Cargos Using Quantitative Mass Spectrometry. *Molecular & Cellular Proteomics*, 12:664-678, PMCID: PMC3591659

c) Landry-Voyer AM, Bilodeau S, Port SA, Rouleau C, Boisvert FM, Kehlenbach RH, and Bachand F. (2016) Human PDCD2L is an export substrate of CRM1 that associates with 40S ribosomal subunit precursors, *Molecular and cellular biology*, 36(24):3019-3032, PMCID: PMC5126290

3. Templating of the SNARE complex by the SM protein Vps33.

To test and extend our model that the SM protein Vps33 acts as a molecular chaperone to template the formation of productive SNARE complexes, we used single-molecule force microscopy. Data are consistent with the model that the SM protein Vps33 catalyzes the step-wise assembly of the four SNARE motifs into a four-helix bundle via a defined pathway. First, the ‘half-zippered’ template complex forms. Second, the Qb- and Qc-SNAREs recognize and bind to the template complex. Finally, full zippering of the SNARE complex displaces the SM protein template. Analogous experiments using the neuronal SM protein Munc18-1 and the SNAREs required for neurotransmitter release lead to a similar conclusion, suggesting that the templating mechanism is conserved among SM proteins.

a) Jiao J, He M, Port SA, Baker RW, Xu Y, Qu H, Xiong Y, Wang Y, Jin H, Eisemann TJ, Hughson FM, Zhang Y (2018) Munc18-1 catalyzes neuronal SNARE assembly by templating SNARE association, *eLife* 2018;7:e41771, PMCID: PMC6320071

Conference contributions

11/2018 2018 Purdue Cryo-EM Symposium
07/2018 Princeton-Nature Conference "The Frontiers in Electron Microscopy for the Physical and Life Sciences", Princeton, NJ, USA (Poster, Talk)
12/2017 ASCB-EMBO meeting, Philadelphia, USA (Poster)
07/2017 Gordon Conference on Molecular Membrane Biology, Andover, NH, USA (Poster)
06/2017 FEBS/ EMBO Advanced Lecture Course “Molecular Architecture, Dynamics and Function of Biomembranes”, Cargèse, France (Poster)
09/2015 EMBO meeting, Birmingham, UK (Poster)
08/2014 Nuclear Organization and Function, Cold Spring Harbor, USA (Talk)
07/2014 Nuclear Envelope “Life at the Edge”, Potsdam, Germany (Poster)
02/2013 Molecular machines in RNA processing, translation and transport, Göttingen, Germany (Poster)

Complete List of Published Work in MyBibliography:

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

Ongoing Research Support

NA

Completed Research Support

5/2017-4/2019 Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) research fellowship PO 2195/1-1

BIOGRAPHICAL SKETCH

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NAME: Hughson, Frederick M.

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

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Yale University	B.S.	05/1984	Molecular Biophysics & Biochemistry
Stanford University School of Medicine	Ph.D.	08/1990	Biochemistry
Harvard University	Postdoctoral	10/1994	Biochemistry

A. Personal Statement

My group uses diverse methods, and especially X-ray crystallography, to study fundamental problems in cell biology. Our primary focus has been on intracellular trafficking in eukaryotes. Several of our current projects involve structural and mechanistic studies of large multi-subunit protein complexes that orchestrate the docking and fusion of transport vesicles. These complexes guide cargo-laden vesicles to their destinations and coordinate the activities of other components of the trafficking machinery, including the SNARE proteins that catalyze membrane fusion itself. We have also started studying the roles of Sec1/Munc18 (SM) proteins in the assembly of fusogenic SNARE complexes. Our crystallographic studies of SM–SNARE complexes recently led to a new model for SM protein mechanism.

I am well-qualified for these studies based on my graduate work on protein folding, my postdoctoral work determining the crystal structure of influenza hemagglutinin in its active, low-pH induced conformation, and my over 20 years as a structural biologist at Princeton, during which we have determined almost 40 X-ray crystal structures. I have extensive experience administering a productive laboratory as demonstrated by our funding and publication records. I have trained over 25 Ph.D. students and postdoctoral fellows, many of whom have gone on to tenure-track faculty positions (and four of whom are now tenured). I maintain a highly interactive environment where lab members are encouraged to share ideas, expertise, and reagents. Moreover, I continue to perform laboratory research, ensuring that I remain connected and accessible to my trainees.

Publications most relevant to the proposed research:

1. Nicholson, K.L., Munson, M., Miller, R.B., Filip, T.J., Fairman, R., and Hughson, F.M. (1998) Regulation of SNARE complex assembly by an N-terminal domain of the t-SNARE Sso1p. Nature Struct Biol 5, 793-802.
2. Baker, R.W., Jeffrey, P.D., and Hughson, F.M. (2013) Crystal structures of the Sec1/Munc18 (SM) protein Vps33, alone and bound to the homotypic fusion and vacuolar protein sorting (HOPS) subunit Vps16. PLoS One 8, e67409. PMCID: PMC3693963
3. Baker, R.W., Jeffrey, P.D., Zick, M., Phillips, B.P., Wickner, W.T., and Hughson, F.M. (2015) A direct role for the Sec1/Munc18-family protein Vps33 as a template for SNARE assembly. Science 349, 1111-1114. PMCID: PMC4727825

4. Jiao, J., He, M., Port, S.A., Baker, R.W., Xu, Y., Qu, H. Xiong, Y., Wang, Y. Jin, H., Eisemann, T.J., Hughson, F.M.*, and Zhang, Y.* (2018) Munc18-1 catalyzes neuronal SNARE assembly by templating SNARE association. eLife, e41771. PMCID: PMC6320071 (*Corresponding authors)

B. Positions and Honors

Positions and Employment

1984-1990	Graduate Fellow, Stanford Department of Biochemistry Robert L. Baldwin, advisor. Research: Protein folding
1990-1994	Postdoctoral Fellow, Harvard Dept. of Biochemistry & Molecular Biology Don C. Wiley, advisor. Research: X-ray crystal structure of low-pH hemagglutinin
1994-2002	Assistant Professor of Molecular Biology, Princeton University
2002-2009	Associate Professor of Molecular Biology, Princeton University
2006-2015	Director, Princeton/HHMI Undergraduate Science Education Program
2009-	Professor of Molecular Biology, Princeton University

Other Experiences and Professional Memberships

1999-	Member of more than ten NIH Special Emphasis Panels
2003	Ad hoc reviewer, NIH CDF-4 Study Section
2003-	Editorial Board, PLoS Biology
2008	Ad hoc reviewer, NIH MSFB Study Section
2010-17	Member, AHA Peer Review Committee (PC1: Basic Cell Biology)
2011-17	Member, ASBMB Education and Professional Development Committee
2015	Chair, Gordon Research Conference on Molecular Membrane Biology
2015-	Board of Reviewing Editors, Science

Honors

1984-87	NSF Pre-doctoral Fellowship
1990-94	Helen Hay Whitney Postdoctoral Fellowship
1995-98	Searle Scholar
1995-97	Beckman Young Investigator
2015	President's Award for Distinguished Teaching, Princeton University

C. Contributions to Science

1. The mechanisms by which cells distribute materials among intracellular organelles, and execute exo- and endocytosis, are of fundamental importance in cell biology. SNARE proteins, discovered in 1993 by Jim Rothman's group, play central roles in these processes, yet when I started my lab in 1994 little was known about the structures and conformational dynamics of this protein family. Our early work, focused on exocytic SNAREs in yeast and neurons, helped to fill this gap by (1) characterizing the pathway of SNARE assembly in vitro and its relationship to protein folding, (2) showing that SNARE assembly is kinetically controlled by the requirement for a conformational change in a key SNARE protein, (3) investigating this conformational change in energetic and structural terms, and (4) characterizing SNARE assembly in vivo.

    a. Nicholson, K.L., Munson, M., Miller, R.B., Filip, T.J., Fairman, R., and Hughson, F.M. (1998) Regulation of SNARE complex assembly by an N-terminal domain of the t-SNARE Sso1p. Nature Struct Biol 5, 793-802.
    b. Lerman, J.C., Robblee, J., Fairman, R., and Hughson, F.M. (2000) Structural analysis of the neuronal SNARE protein syntaxin-1A. Biochemistry 39, 8470-8479.
    c. Munson, M., Chen, X., Cocina, A.E., Schultz, S.M., and Hughson, F.M. (2000) Interactions within the yeast t-SNARE Sso1p that control SNARE assembly. Nature Struct Biol 7, 894-902.
    d. Munson, M. and Hughson, F.M. (2002) Conformational regulation of SNARE assembly and disassembly in vivo. J Biol Chem 277, 9375-9381.

2. In a second phase of our work on the machinery of vesicle trafficking, we turned to the multisubunit 'tethering' complexes (MTCs) that interact with SNAREs to orchestrate vesicle docking and fusion. We

have worked on three MTCs, one of which (the Dsl1 complex) is described here and another of which (the COG complex) is described in the next section. By combining single particle EM (carried out in collaboration with Tom Walz) and X-ray crystallography, we were able to propose a nearly complete atomic-resolution model for the hetero-trimeric, 260-kDa Dsl1 complex. Our results strongly suggested that even the simplest MTC is capable of orchestrating vesicle capture, uncoating, and fusion.

a. Tripathi, A., Ren, Y., Jeffrey, P.D., Hughson, F.M. (2009) Structural characterization of Tip20p and Dsl1p, subunits of the Dsl1p vesicle tethering complex. Nature Struct Mol Biol 16, 114-123. PMCID: PMC2635920
b. Ren, Y., Yip, C., Tripathi, A., Huie, D., Jeffrey, P.D., Walz, T., and Hughson, F.M. (2009) Structural basis for vesicle tethering by the Dsl1p complex. Cell 139, 1119-1129. PMCID: PMC2806190
c. Rogers, J.V., McMahon, C., Baryshnikova, A., Hughson, F.M., and Rose, M.D. (2014) ER-associated retrograde SNAREs and the Dsl1 complex mediate an alternative, Sey1p-dependent homotypic ER fusion pathway. Mol Biol Cell 25, 3401-3412. PMCID: PMC4214786
d. Suckling, R.J., Poon, P.P., Travis, S.M., Hughson, F.M., Evans, P.R., Duden, R., and Owen, D.J. (2015) Structural basis for the binding of tryptophan-based motifs by delta-COP. Proc Natl Acad Sci USA 112, 14242-14247. PMCID: PMC4655537

3. Another and much larger multisubunit tethering complex (MTC), the hetero-octameric COG complex (650 kDa), was initially isolated on the basis of its ability to stimulate an in vitro Golgi transport assay. Subsequent work strongly implied that COG functions to tether vesicles carrying cargo between Golgi cisternae. It therefore plays a key role in maintaining the proper distribution of glycosylation enzymes and other materials within the Golgi. As a consequence, COG defects can lead to fatal glycosylation disorders. To provide a foundation for understanding COG function in mechanistic terms, we have: (1) used biochemical and single particle EM-based approaches to determine the molecular architecture of the yeast and mammalian COG complexes, (2) elucidated the structural basis for human glycosylation disorders caused by mutations in several COG subunits, and (3) used X-ray crystallography to reveal the structural basis for the subunit interactions that hold COG (and other multisubunit tethering complexes) together.

a. Richardson, B.C., Smith, R.D., Ungar, D., Nakamura, A., Jeffrey, P.D., Lupashin, V.V., and Hughson, F.M. (2009) Structural basis for a human glycosylation disorder caused by mutation of the *COG4* gene. Proc Natl Acad Sci USA 106, 13329-13334. PMCID: PMC2716380
b. Lees, J.A., Yip, C.K., Walz, T., and Hughson, F.M. (2010) Molecular organization of the COG vesicle tethering complex. Nature Struct Mol Biol 17, 1292-1297. PMCID: PMC3113405
c. Ha, J.Y., Pokrovskaya, I.D., Climer, L.K., Shimamura, G.R., Kudlyk, T., Jeffrey, P.D., Lupashin, V.V., and Hughson, F.M. (2014) Cog5-Cog7 crystal structure reveals interactions essential for the function of a multisubunit tethering complex. Proc Natl Acad of Sci USA 111, 15762-15767. PMCID: PMC4226102
d. Ha, J.Y., Chou, H.T., Ungar, D., Yip, C.K., Walz, T., and Hughson, F.M. (2016) Molecular architecture of the complete COG tethering complex. Nature Struct Mol Biol 23, 758-760. PMCID: PMC4972656

4. In 2002, we initiated a collaboration with my Princeton colleague Bonnie Bassler to study bacterial cell-cell communication, or quorum sensing. Our work has elucidated (1) the structures of signal molecules used to mediate quorum sensing, (2) structures of the bacterial receptors for these signal molecules, and (3) mechanisms whereby quorum sensing can be modulated by synthetic molecules. The last of these represents a new approach to the discovery and optimization of broad spectrum anti-bacterial compounds.

a. Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczer, I., Bassler, B.L., and Hughson, F.M. (2002) Structural identification of a bacterial quorum sensing signal containing boron. Nature 415, 545-549.
b. Neiditch, M.B., Federle, M.J., Pompeani, A.J., Kelly, R.C., Swem, D.L., Jeffrey, P.D., Bassler, B.L., and Hughson, F.M. (2006) Ligand-induced asymmetry in histidine sensor kinase complex regulates quorum sensing. Cell 126, 1095-1108.
c. Chen, G., Swem, L.R., Swem, D.L., Stauff, D.L., O'Loughlin, C.T., Jeffrey, P.D., Bassler, B.L., and Hughson, F.M. (2011). A strategy for antagonizing quorum sensing. Mol Cell 42, 199-209. PMCID: PMC3982643

d. Boyaci, H., Shah, T., Hurley, A., Kokona, B., Li, Z., Ventocilla, C., Jeffrey, P.D., Semmelhack, M.F., Fairman, R., Bassler, B.L., and Hughson, F.M. (2016) Structure, regulation, and inhibition of the quorum-sensing signal integrator LuxO. PLoS Biol 14, e1002464. PMCID: PMC4878744

Complete List of Published Work in MyBibliography:
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D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 GM071574 Hughson (PI) 3/1/2005 – 2/28/2021
NIH - NIGMS
Structural Analysis of Membrane Tethering and Fusion Proteins
One goal of this study is structure-function analyses of two membrane tethering complexes – HOPS and Dsl1 – and their interactions with other trafficking proteins, particularly SNAREs and vesicle coat proteins. The other goal is test our hypothesis that SM proteins function as templates to catalyze SNARE assembly, and to explore the ramifications of this hypothesis.