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, single particle cryo-EM and X-ray crystallography methods to a complex of nuclear transport receptor, transport cargo 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 conduct postdoctoral studies on 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.

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 and 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 generated a crystal structure of a FG-repeat-containing fragment of Nup214 bound to a CRM1 export complex. 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

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:

https://www.ncbi.nlm.nih.gov/sites/myncbi/1Xy_tr6VnSX5N/bibliography/48341369/public/?sort=date&direction=descending

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

Ongoing Research Support

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

Completed Research Support

NA.