BIOGRAPHICAL SKETCH

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NAME: Steimle, Stefan Markus

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POSITION TITLE: Postdoctoral Researcher, Biology

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
University of Freiburg, Germany	M.Sc. (foreign)	09/2009	Chemistry
University of Freiburg, Germany	Ph.D.	07/2014	Biochemistry
University of Pennsylvania, Philadelphia	postdoctoral	07/2020	Biochemistry & Structural Biology

A. Personal Statement

I have the experience, previous training, expertise & motivation necessary to take great advantage of the proposed use of the Chameleon system. My early work with large membrane protein complexes, traditionally the most difficult samples in structural biology, put me in the position to successfully purify challenging samples and prepare them for structural studies, a logical extension of my previous work on the functional characterization of respiratory enzymes. Over the last 3 years I worked my way into the field of cryo-EM, collaborating intensively with established experts in the field and core facility specialists. I mastered a variety of techniques ranging from sample preparation and initial screening to data collection and computational analysis, and with the help of my collaborators, I was able to obtain a medium resolution (6-7Å) structure of our membrane protein complex. While we were able to optimize the purification procedure to obtain a pure and almost homogenous sample, the vitrification process remains a bottleneck in our study. We believe that the advantages of the new Chameleon technology provide an excellent opportunity for us to better control the vitrification of our sample and reach a higher resolution.

B. Positions and Honors

Positions and Employment

2010-2014 Research Associate and Ph.D. candidate, Department of Biochemistry, University of Freiburg, Germany
2015- Postdoctoral Researcher, Department of Biology, University of Pennsylvania, Philadelphia, PA

Honors

2012 Ph.D. fellowship, awarded by the International Graduate Academy, University of Freiburg, Germany

C. Contributions to Science

1. My early research as a PhD candidate focused on respiratory complex I, a multi-subunit membrane protein complex important in health and disease. Dysfunctions of complex I are linked to severe neurological disorders such as Alzheimer's and Parkinson's Disease and a detailed understanding of the enzyme's mechanism is essential for prevention and proper treatment. I used site-directed mutagenesis, protein purification and functional studies to shed light on the mechanism of proton translocation, highlighting the importance of specific residues or subunits within the enzyme. The results of my work are documented in several peer-reviewed publications.

a. Steimle, S., Bajzath, C., Dörner, K., Schulte, M., Bothe, V. & Friedrich, T. (2011). Role of subunit NuoL for proton translocation by respiratory complex I. *Biochemistry* **50**, 3386-3393.
b. Steimle, S., Erhardt, H., Muders, V., Pohl, T., Walter, J. & Friedrich, T. (2012). Disruption of individual *nuo*-genes leads to the formation of partially assembled NADH:ubiquinone oxidoreductase (complex I) in *Escherichia coli. Biochim. Biophys. Acta* **1817**, 863-871.
c. Steimle, S., Willistein, M., Hegger, P., Janoschke, M., Erhardt, H. & Friedrich, T. (2012). Asp563 of the horizontal helix of subunit NuoL is involved in proton translocation by the respiratory complex I. *FEBS Lett.* **586**, 699-704.
d. Steimle, S., Schnick, C., Burger, E.-M., Nuber, F., Krämer, D., Dawitz, H., Brander, S., Matlosz, B., Schäfer, J., Maurer, K., Glessner, U. & Friedrich, T. (2015). Cysteine scanning reveals minor local rearrangements of the horizontal helix of respiratory complex I during turnover. *Mol. Microbiol.* **98**, 151-161.

2. In my recent NIH-funded postdoctoral work, which is a logical expansion of my previous research, I used my skills surrounding the biochemistry of membrane proteins to study supercomplex assemblies of two additional respiratory enzymes, namely complex III (CIII) and complex IV (CIV). In recent years, there has been mounting evidence that supercomplexes of the respiratory chain play an important role in stabilizing individual components, enhancing catalytic efficiency through substrate channeling and minimizing the production of reactive oxygen species. We used a genetic fusion of the cytochrome c reductase (CIII) and cytochrome c oxidase (CIV) and, in addition to native PAGE analysis and functional assays, I was able to purify several versions of the construct and obtain the cryo-EM structure of the entire complex. In collaboration with Dr. Kenji Murakami, a well-known single particle cryo-EM specialist at the Perelman School of Medicine (University of Pennsylvania), we are currently working on the final refinement of the structure which will provide an important insight in cofactor binding and the mechanism of electron transfer. This is especially interesting because it is the first structure of a respiratory supercomplex containing a cbb3-type cytochrome c oxidase (CIV), a version of the enzyme that is unique to a number of bacterial pathogens such as *Helicobacter pylori* and therefore a potential drug target.
In addition to leading this project and pioneering cryo-EM in our lab, I made various contributions to publications resulting from work done in our group and in collaboration with other researchers.

a. Francia, F., Malferrari, M., Lanciano, P., Steimle, S., Daldal, F. & Venturoli, G. (2016). The cytochrome b Zn binding amino acid residue histidine 291 is essential for ubihydroquinone oxidation at the Q_o site of bacterial cytochrome bc_1. *Biochim. Biophys. Acta* **1857**, 1796-1806.
b. Verissimo, A.F., Khalfaoui-Hassani, B., Hwang, J., Steimle, S., Selamoglu, N., Sanders, C., Khatchikian, C.E. & Daldal, F. (2017). The thioreduction component CcmG confers efficiency and the heme ligation component CcmH ensures stereo-specificity during cytochrome c maturation. *J. Biol. Chem.* **292**, 13154-13167.
c. Zhang, Y., Blaby-Haas, C.E., Steimle, S., Verissimo, A.F., Garcia-Angulo, V.A., Koch, H.G., Daldal, F. & Khalfaoui-Hassani, B. (2019). Cu transport by the extended family of CcoA-like transporters (CalT) in Proteobacteria. Sci. Rep. **9**:1208.