BIOGRAPHICAL SKETCH

NAME: Pleiner, Tino

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING:

INSTITUTION AND LOCATION	DEGREE	Completion Date MM/YYYY	FIELD OF STUDY
University of Leipzig, Germany	BS	08/2010	Biochemistry
University of Göttingen, Germany	MS	03/2012	Molecular Biology
University of Göttingen, Germany / Max Planck Institute for Multidisciplinary Sciences	PhD	05/2016	Molecular Biology
Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany	Postdoctorate	10/2017	Molecular Biology
California Institute of Technology, Pasadena, CA, USA	Postdoctorate	05/2023	Molecular and Cellular Biology

A. Personal Statement

I am a new Assistant Professor in the Department of Molecular and Cellular Physiology at Stanford University's School of Medicine. Through my education and training I have acquired a broad interdisciplinary background in molecular and cellular biology, as well as protein engineering. My research is focused on how human cells balance protein biogenesis and degradation to maintain protein homeostasis and how these processes fail in diseases like cancer, neurodegeneration and ageing. A particular focus is on the mechanisms of membrane protein homeostasis. I have laid the groundwork for these inquiries during my postdoctoral work. I joined the new lab of Rebecca Voorhees at Caltech as her first postdoc shortly after she started her own lab. Here, I quickly established a novel line of research into the mechanism of membrane protein biogenesis and assembly in human cells. This led to the first ever structure of an essential membrane protein biogenesis factor called the ER membrane protein complex (EMC) (Pleiner et al., 2020, *Science*). Using this structure I could gain first mechanistic insights into EMC function and assembly in cells (Pleiner et al., 2021, *Mol Cell*). My independent lab will additionally leverage my unique background in the engineering of small single domain antibodies derived from alpacas called nanobodies (Stevens et al., 2023, bioRxiv). As a PhD student in the lab of Dirk Görlich at the Max Planck Institute of Multidisciplinary Sciences, I independently established a highly streamlined workflow to generate nanobodies from alpacas using an on-site alpaca farm that was built expressly for my PhD project. Exploiting this prime access to the nanobody technology, I pioneered several now widely used methods to engineer nanobodies as precision tools for cell biology and super-resolution microscopy (Pleiner et al., 2018, *JCB*).

Selected publications:
(*denotes equal contribution co-first- and † denotes co-corresponding authorship)

1. **Pleiner, T.***, Tomaleri, G.P. *, Januszyk, K. *, Inglis, A.J., Hazu, M. and Voorhees, R.M. (2020) Structural basis for membrane insertion by the human ER membrane protein complex. *Science*, **369**, 433-436.
2. **Pleiner, T.**, Hazu, M., Tomaleri, G.P., Januszyk, K., Oania, R.S., Sweredoski, M.J., Moradian, A., Guna, A. and Voorhees, R.M. (2021) WNK1 is an assembly factor for the human ER membrane protein complex. *Mol Cell*, **81**, 2693-2704.e12.
3. Stevens, T.A., Tomaleri, G.P., Hazu, M., Wei, S., Nguyen, V.N., DeKalb, C., Voorhees, R.M.† and **Pleiner, T.**† (2023) A nanobody-based strategy for rapid and scalable purification of native human protein complexes. *Nat Protoc.* 2024 Jan;19(1):127-158. doi: 10.1038/s41596-023-00904-w.
4. **Pleiner, T.**†, Bates, M.† and Görlich, D.† (2018) A toolbox of anti-mouse and anti-rabbit IgG secondary nanobodies. *J Cell Biol*, **217**, 1143-1154.

B. Positions and Honors

Positions and Employment

2023 – Present Assistant Professor, Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA, USA (start date: 06/01/2023)

Professional Memberships

2023 – Present Member, American Society for Biochemistry and Molecular Biology (ASBMB)
2008 – Present Member, German Society for Biochemistry and Molecular Biology (GBM)

Honors

2020 Paper: Pleiner et al., 2018, J. Cell Biol. featured in the 65th Anniversary collection of "methods that shaped cell biology" of the Journal of Cell Biology
2019 – 2021 Postdoctoral fellowship of the German research foundation (DFG)
2018 – 2019 Caltech 'Ross' postdoctoral fellowship
2018 Paper: Pleiner et al., 2018, J. Cell Biol. featured in the "The Year in Cell Biology" collection of the Journal of Cell Biology
2018 Animal welfare prize by German Federal Ministry for Food and Agriculture ($25,000, shared with Dr. Dirk Görlich)
2016 Poster prize at the '13th Horizons in Molecular Biology Symposium', Göttingen, Germany
2014 Selected participant of the '64th Lindau Nobel Laureate Meeting', nominated by Göttingen Graduate School GGNB
2010 – 2011 International Max Planck Research School (IMPRS) fellowship
2010 Study prize for best Bachelor of Science Biochemistry degree, University of Leipzig
2009 – 2012 Fellowship of the German Academic Scholarship Foundation (Studienstiftung des deutschen Volkes)

C. Contributions to Science

1. Membrane proteins make up ~20 % of the human proteome and fulfill essential cellular functions in the regulation of cellular information and nutrient exchange, which makes them attractive drug targets. Despite their importance for normal cellular physiology as well as disease, little is known about how many biomedically relevant membrane proteins are inserted, folded and assembled at the endoplasmic reticulum (ER). Biogenesis factors called insertases facilitate membrane protein insertion into the lipid bilayer. The highly conserved 9-subunit ER membrane protein complex (EMC) has recently emerged as a major insertase of many essential endogenous (e.g. GPCRs, ion channels and transporters), as well as viral membrane proteins. Due to the absence of structural and functional data at the time, it was unclear how the EMC fulfills these critical roles in membrane protein biogenesis.

   During my postdoctoral work, I adapted a nanobody-based purification strategy that I developed during my PhD to purify the human EMC and closely collaborated with other Voorhees lab members to solve its first ever structure. Using this structure, I designed cell-based fluorescent reporter assays to gain first mechanistic insights into EMC's insertase function. I found that the EMC uses local membrane thinning, as well as a conserved positively charged intramembrane vestibule to decrease the energetic barrier for TMD insertion into the lipid bilayer. My work revealed general mechanistic insights into the conserved process of transmembrane domain (TMD) insertion that are broadly applicable to evolutionarily related insertases found across all kingdoms of life. I prepared the sample for structural analysis and performed the majority of the *in vitro* biochemistry, as well as all cell-based functional assays. I wrote the manuscript with Rebecca Voorhees.

   a. **Pleiner, T.***, Tomaleri, G.P.*, Januszyk, K.*, Inglis, A.J., Hazu, M. and Voorhees, R.M. (2020) Structural basis for membrane insertion by the human ER membrane protein complex. *Science*, **369**, 433-436.

2. Subcellular compartmentation is a hallmark of eukaryotic cells and requires the accurate sorting of thousands of nascent proteins into specific organelles. Sorting of the ~5,000 human membrane proteins in particular must be tightly regulated to prevent their aggregation in the cytosol. Many membrane proteins do not use cleavable signal sequences and instead are sorted based on subtle sequence differences – e.g. positive

charges adjacent to transmembrane domains. How these sequence features are interpreted to ensure accurate sorting has been a longstanding open question in cell biology.
Biophysical similarities lead to mistargeting of mitochondrial membrane proteins to the ER, where they are delivered to the ER membrane protein complex (EMC) insertase. Using a combination of mutagenesis and site-specific crosslinking *in vitro* and in cells revealed that the EMC uses a positively charged intramembrane vestibule as a selectivity filter to limit mis-insertion of mitochondrial outer membrane proteins with positively charged domains into the ER membrane via charge repulsion. These finding established the membrane insertion step as a new level of regulatory control that determines accurate protein localization in human cells.

The topology of all multi-spanning membrane proteins is established by the insertion of their first TMD. The EMC is the first insertase to access all nascent membrane proteins and thus makes critical decisions over membrane protein topology. I discovered that the EMC selectivity filter enforces correct topology and the so-called positive-inside rule by blocking translocation of frequently positively charged cytosolic domains. This work redefined the EMC as a guardian of the ER. The EMC protects compartment identity by limiting mis-insertion of mitochondrial membrane proteins and thus contributes to protein sorting fidelity. It also ensures accurate membrane protein topology and thus limits misfolding and aggregation. This work was carried out in close collaboration with two talented graduate students, Giovani Pinton Tomaleri and Masami Hazu, who I trained and helped mentor over the past 4 years. I wrote the manuscript with Rebecca Voorhees and input from all team members.

a. **Pleiner, T.***, Hazu, M.*, Tomaleri, G.P.*, Nguyen, V.N., Januszyk, K. and Voorhees, R.M. (2023) A selectivity filter in the EMC limits protein misinsertion at the ER. *J. Cell Biol* **222**, e202212007.

3. Nearly 50% of all human proteins need to assemble into complexes in order to function. How are newly synthesized unassembled subunits routed towards a productive assembly path and prevented from aggregation or non-specific off-pathway interactions in the crowded cytosol? Proteomics data clearly showed that protein complex assembly is highly regulated, yet the molecular mechanisms remained poorly characterized, especially for the assembly of membrane proteins.

   The EMC proved to be a tractable model system to better understand how membrane protein complex assembly and quality control are regulated. I discovered that the essential kinase WNK1 moonlights as an EMC assembly factor and characterized its requirement using *in vitro* reconstitution and cell-based stability assays. I found that WNK1 uses a conserved amphipathic helix to prevent E3 ubiquitin ligase binding and thus premature degradation of the newly synthesized soluble EMC subunit EMC2 by shielding an exposed hydrophobic inter-subunit interface. WNK1's role in EMC assembly revealed several general properties of an assembly factor that apply broadly across the proteome. I performed most of the experiments in the paper with help from graduate student Masami Hazu, who I closely supervised. I wrote the manuscript with Rebecca Voorhees and input from all team members.

   a. **Pleiner, T.**, Hazu, M., Tomaleri, G.P., Januszyk, K., Oania, R.S., Sweredoski, M.J., Moradian, A., Guna, A. and Voorhees, R.M. (2021) WNK1 is an assembly factor for the human ER membrane protein complex. *Mol Cell*, **81**, 2693-2704.e12.

4. Nanobodies are small (~13 kDa) single-domain antibodies derived from camelids like alpacas that can be expressed recombinantly in bacteria, as well as inside living human cells. During my PhD, I established one of the few academic facilities worldwide for the generation of alpaca-derived nanobodies. The Görlich lab did not work on nanobodies before I joined and I therefore trained myself independently in all the necessary techniques. I supervised a technical assistant that helped with immunization and nanobody library generation. As part of my own work and in collaboration, I generated nanobodies against a wide variety of targets. In the Görlich lab, I used nanobodies as tools to study nucleocytoplasmic transport of proteins through nuclear pore complexes (NPCs): first, as crystallization chaperones for an essential heterotrimeric complex that lines the NPC transport channel (Chug et al., 2015, Science); and second, as intracellular inhibitors of the nuclear transport receptor (NTR) Exportin 7, which allowed us to demonstrate that this NTR could unexpectedly carry-out bidirectional protein transport (Aksu* and Pleiner* et al., 2018, JCB).

   To facilitate the structural and biochemical characterization of NPC components and NTRs and to provide purified protein samples for nanobody generation, I developed a highly efficient strategy for the native purification of endogenous proteins using nanobodies that can be rapidly cleaved off affinity resin using a highly active orthogonal SUMO protease (Pleiner et al., 2015, eLife). I conceptualized the strategy with Dirk Görlich, carried out all experiments and wrote the paper with Dirk Görlich.

Since this method delivered high yields and purity, I later adapted it during my postdoc to the anti-GFP nanobody and used it to isolate the EMC for structural analysis by cryo-EM (Pleiner et al., 2020, Science). Following many plasmid requests and successful implementation of this flexible GFP-based purification technique in other labs at Caltech, I was motivated to assemble a protocol paper with the help of Voorhees lab graduate student Taylor Stevens whom I trained and supervised in this approach (Stevens et al., 2023, bioRxiv). I conceptualized the technology and wrote the manuscript with input from Rebecca Voorhees and Taylor Stevens.

a. Chug, H., Trakhanov, S., Hülsmann, B.B., **Pleiner, T.** and Görlich, D. (2015) Crystal structure of the metazoan Nup62•Nup58•Nup54 nucleoporin complex. *Science*, **350**, 106-110.
b. Aksu, M.*, **Pleiner, T.***, Karaca, S., Kappert, C., Dehne, H.J., Seibel, K., Urlaub, H., Bohnsack, M.T. and Görlich, D. (2018) Xpo7 is a broad-spectrum exportin and a nuclear import receptor. *J Cell Biol*, **217**, 2329-2340.
c. **Pleiner, T.**, Bates, M., Trakhanov, S., Lee, C.T., Schliep, J.E., Chug, H., Böhning, M., Stark, H., Urlaub, H. and Görlich, D. (2015) Nanobodies: site-specific labeling for super-resolution imaging, rapid epitope-mapping and native protein complex isolation. *Elife*, **4**, e11349.
d. Stevens, T.A., Tomaleri, G.P., Hazu, M., Wei, S., Nguyen, V.N., DeKalb, C., Voorhees, R.M.† and **Pleiner, T.**† (2023) A nanobody-based strategy for rapid and scalable purification of native human protein complexes. *bioRxiv*, https://doi.org/10.1101/2023.03.09.531980.

5. Since nanobodies are ~10x smaller than conventional antibodies they are powerful tools for precision localization in super-resolution microscopy. One key requirement for this application was the ability to attach multiple bright fluorophores to a single nanobody without interfering with antigen binding. At the time nanobodies were purified and labeled with fluorophores using the same protocols as their much larger conventional counterparts. These approaches often even led to nanobody inactivation or aggregation and consequently did not allow multi-dye labeling.

   By leveraging their unique ability to be engineered and expressed recombinantly, I developed a site-specific fluorescent labeling strategy that maximizes nanobody brightness, while maintaining specificity and stability (Pleiner et al., 2015, eLife). In collaboration with the group of Stefan Hell, I used nanobodies as ultra-small immunolabels for super-resolution microscopy (Pleiner et al., 2015, eLife; Göttfert et al., 2017, PNAS; Pleiner et al., 2018, JCB). My labeling strategy proved essential for this and later also allowed me to generate bright anti-mouse and rabbit IgG 'secondary nanobodies' that could rival the signal amplification effect of conventional polyclonal secondary antibodies (Pleiner et al., 2018, JCB). The resulting nanobody plasmids have been distributed by Addgene to hundreds of labs worldwide (Blue Flame Award). Multiple antibody companies also started to provide secondary nanobodies for imaging as a result. This study was selected for a special collection of the Journal of Cell Biology (JCB65: Methods) and awarded with the 'animal welfare prize' of the German government. I carried out all nanobody phage display selections, as well as nanobody characterization experiments for all papers. I wrote both first-author papers with Dirk Görlich. I am a co-corresponding author on the IgG nanobody paper in JCB.

   a. **Pleiner, T.**, Bates, M., Trakhanov, S., Lee, C.T., Schliep, J.E., Chug, H., Böhning, M., Stark, H., Urlaub, H. and Görlich, D. (2015) Nanobodies: site-specific labeling for super-resolution imaging, rapid epitope-mapping and native protein complex isolation. *Elife*, **4**, e11349.
   b. Göttfert, F., **Pleiner, T.**, Heine, J., Westphal, V., Görlich, D., Sahl, S.J. and Hell, S.W. (2017) Strong signal increase in STED fluorescence microscopy by imaging regions of subdiffraction extent. *Proc Natl Acad Sci U S A*, **114**, 2125-2130.
   c. **Pleiner, T.**†, Bates, M.† and Görlich, D.† (2018) A toolbox of anti-mouse and anti-rabbit IgG secondary nanobodies. *J Cell Biol*, **217**, 1143-1154.

Complete List of Published Work in MyBibliography:
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