BIOGRAPHICAL SKETCH

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NAME: Tom A. Rapoport

eRA COMMONS USER NAME (credential, e.g., agency login): TomRap1

POSITION TITLE: Professor of Cell 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
Humboldt-University, Berlin, Germany	PH.D.	06/1972	Chemistry/ Biochemistry
Humboldt-University, Berlin, Germany	Habilitation	07/1977	Biochemistry

A. Personal Statement

Our laboratory is interested in how organelles in cells receive specific set of proteins, how they discard misfolded proteins to maintain their function, and how they achieve their characteristic shape. We are particularly interested in the endoplasmic reticulum (ER), an organelle found in all eukaryotic cells. A large number of proteins are translocated across or integrated into the ER membrane, from where they can be transported to other organelles, such as the Golgi apparatus, the plasma membrane, or lysosomes. The mechanism of translocation is conserved in all organisms. My lab has provided important insights into the mechanism of protein translocation. We have also extensively worked on the mechanism by which misfolded ER proteins are retro-translocated from the ER into the cytosol for degradation (ERAD). More recently, we became interested in the molecular mechanism by which proteins are imported into peroxisomes. Another major effort of the lab concerns the mechanism by which the ER is shaped. I have used these projects to train a large number of students and postdocs, many of whom now occupy faculty positions in leading institutions.

Ongoing and recently completed projects that I would like to highlight include:

R01 GM052586 Rapoport (PI) **05/01/1995-05/31/2027**
Protein Transport Across Membranes
The major goal of this project is to understand in mechanistic terms how proteins are transported across membranes.

R01HL150520 Rapoport (PI) **02/15/2020-01/21/2025**
Function and application of lung surfactant proteins
Lung surfactant is a mixture of phospholipids and proteins, which is essential for breathing. The most important protein component is surfactant protein B (SP-B). The goal of this project is to define the molecular mechanism by which lung SP-B functions in respiration, and to develop an optimized surfactant mixture that could ultimately be used as a therapeutic for the treatment of Acute Respiratory Stress Syndrome (ARDS).

Howard Hughes Medical Institute (HHMI) **07/16/1997-present**
Investigator
Dr. Rapoport's salary and benefits, and those of five postdoctoral associates (Junqiao Jia, Michal Skowyra, Kaiku Uegaki, Dingwei He and Christopher Nardone), one research specialist (Yoko

Shibata), a research technician (Shashank Rao) and an administrative assistant (Lorna Fargo) are paid by HHMI. There is no scientific overlap with regard to HHMI funded projects.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments:

2024-present **Endowed Chair appointment as Don W. Fawcett Professorship of Molecular Cell Biology** in the Dept. of Cell Biology at the Faculty of Medicine of Harvard University
1997-present **Investigator**, Howard Hughes Medical Institute, Harvard Medical School, Boston, USA
1995-1997 **Professor**, Harvard Medical School, Boston, USA
1991-1994 **Professor**, Max-Delbrück-Center, Department of Cell Biology, Berlin, Germany
1985-1990 **Professor**, Central Institute for Molecular Biology, Academy of Science, Berlin, GDR
1972-1985 **Research Associate**, Central Institute for Molecular Biology, Academy of Science, Berlin,GDR

Professional Memberships - German Biochemical Society; Academy of Sciences; Academia Europaea; EMBO; Biophysical Society; American Society for Cell Biology; American Association for the Advancement of Science; International Advisory Board of the Göttingen Graduate School for Neurosciences and Molecular Biosciences (GGNB); External Scientific Member of the Max Planck Institute for Biophysical Chemistry (Karl Friedrich Bonhoeffer Institute) in Göttingen; American Society for Biochemistry and Molecular Biology (ASBMB); MDIBL Scientific Advisory Board (since April, 2021).

Major Committee Assignments

2017	ASCB's 2017 EB Wilson Medal Selection Committee
2017	Molecular Mechanistic Biology (MMB) Steering Committee
2015	Organizer, 2015 Membrane Biology Symposium, Harvard Medical School, Dept. of Cell Biology (meeting date: November 4, 2015)
2015-present	Associated Faculty Member, Microbial Sciences Initiative (MSI), Harvard Medical School
2013-2014	Organizing Committee, EMBO meeting on ER Functions (meeting dates: October 26-31, 2014)
2013	Co-Organizer, EMBO Workshop "2nd International symposium on Membrane shaping and remodeling by proteins" (meeting dates: May 16-19, 2013)
2011-present	Co-Organizer, FEBS/EMBO Lecture Course (biennially)
2010	Co-Organizer, Keystone Meeting on "Molecular Basis for Biological Membrane Organization and Dynamics" (meeting dates: January 10-15, 2010)
2007-present	PQE Steering Committee
2007-2008	Standing Committee on Higher Degrees in Systems Biology
1999-2002	NIH study section member
1997-1999	Vice Chair/Chair, Gordon Research Conference on Molecular Membrane Biology

Honors

Mangrove Lecturer, Shenzhen Bay Laboratory (November, 2024)
IUBMB Jubilee Award Lecturer at the EMBO/FEBS Lecture Course in Spetses, Greece (May, 2024)
Robert and Margaret Haselkorn Lecturer, Univ. of Chicago, Mol. Genetics & Cell Biology (April, 2024)
James B. Sumner Lecturer, Cornell University (April, 2024)
Walter Neupert Lecturer, Munich, Germany (June, 2022)
12th SCILLS Lecturer, University of Dundee (June, 2022)
Rodney Porter Memorial Lecturer, University of Oxford, Department of Biochemistry (May, 2018)
Biochemistry IMPACT Lecturer, University of Notre Dame (April, 2018)
Cell Biology Discovery Lecturer, Johns Hopkins University (March, 2018)
Sun-Kee Kim Lecturer for 2017, University of Michigan (October, 2017)
Inaugural ASCB Fellow (2016)
Harvard University Microbial Sciences Initiative associated faculty member (2015)
Carter Wallace Lecturer for 2014-2015, Princeton University (April, 2015)

Institute for Advanced Study (IAS) Visiting Professor, Hong Kong University of Science and Technology (HKUST) (October 1, 2014-September 30, 2016)
Widom Lecturer, Northwestern University (2013)
Mendel Lecturer, Brno, Czech Republic (2013)
Harvard Medical School Excellence in Tutoring Award (2012)
Schleiden-Medaille from the Leopoldina Academy (2011)
Milstein Lecturer at MRC Laboratory of Molecular Biology (LMB) (2011)
Keith R. Porter Lecturer at 50th Annual Meeting of the ASCB (2010)
Van Deenen Medal (2010)
Anatrace Membrane Protein Award (2010)
Distinguished Research Chair Professor in the Center for Biotechnology at National Taiwan University (August 1, 2009 – July 31, 2020)
Clifford Barger Excellence in Mentoring Award (2009)
AAAS Fellow (2007)
Sir Hans Krebs Medal recipient (2007)
Member of the National Academy of Sciences (USA) (2005)
Member of the American Academy of Arts and Sciences (2005)
Max Delbrück Medal recipient (2005)
Otto-Warburg-Medaille of the GBM in the field of Biochemistry (2004)
Member of the Leopoldina Academy (German National Academy of Sciences) (2003)
Rudolf-Virchow-prize (1980)
Johannes-Müller-prize of the Society for Experimental Medicine (1973)

C. Contributions to Science

Development of Metabolic Control Analysis (MCA), a theory that describes the regulation of metabolic networks (together with Reinhart Heinrich); 1972-80

1. How are metabolic pathways and networks regulated? Which role do the various enzymes participating in a pathway have on the overall flux and on the level of individual metabolites? We developed a mathematical theory that ascribes control coefficients to each enzyme. This theory is now called Metabolic Control Analysis (MCA) and is often considered to be the starting point of systems biology.

a. *Heinrich, R. and Rapoport, T. A. (1973) Acta biol. med. germ. 31, 479-494. Linear theory of enzymatic chains; its application for the analysis of the crossover theorem and of the glycolysis of human erythrocytes.*
b. *Heinrich, R. and Rapoport, T. A. (1974) Eur. J. Biochem. 42, 89-95. A linear steady state treatment of enzymatic chains; general properties, control and effector strength.*
c. *Heinrich, R. and Rapoport, T. A. (1974) Eur. J. Biochem. 42, 97-105. A linear steady state treatment of enzymatic chains; critique of the crossover theorem and a general procedure to identify interaction sites with an effector.*

Elucidation of the mechanism of protein translocation (1986-now)

2. How are proteins translocated across or integrated into the endoplasmic reticulum (ER) membrane in eukaryotes or the plasma membrane in prokaryotes? We first introduced a photo-crosslinking approach to identify components that are close to the translocating polypeptide chain. This led to the discovery that Sec61 forms a protein-conducting channel. The conclusion was supported by reconstitution of co-translational translocation with purified components. Other experiments showed that the Sec61 complex recognizes signal sequences and that, together with another membrane protein complex, it mediates post-translational translocation. We showed that post-translational translocation occurs by a ratcheting mechanism with BiP as an ATP-dependent molecular ratchet. In 2004, we were able to determine the first crystal structure of a protein-conducting channel (collaboration with Steve Harrison). In subsequent years we studied the mechanism of post-translational translocation in bacteria. We determined a crystal structure of a SecA-SecY complex, and showed that the ATPase SecA uses a “push-and-slide” mechanism to

move polypeptides. In 2016, we reported the first high-resolution structure of an active translocon, revealing the mechanism of signal sequence recognition. The results of our research are now covered in textbooks.

a. *Görlich, D. and Rapoport, T.A. (1993) Cell 75, 615-630. Protein translocation into proteoliposomes reconstituted from purified components of the ER membrane.*
b. *Matlack, K.E.S., Misselwitz, B., Plath, K., and Rapoport, T.A. (1999) Cell 97, 553-564. BiP acts as a molecular ratchet during posttranslational transport of prepro-α-factor across the ER membrane.*
c. *van den Berg, L., Clemons, W., Collinson, I., Modis, Y., Hartmann, E., Harrison, S.C., and Rapoport, T.A. (2004) Nature 427, 36-44. X-ray structure of a protein-conducting channel.*
d. *Wu, X., Cabanos, C., and Rapoport, T.A. (2019) Nature 566, 136-139. Structure of the post-translational protein translocation machinery of the ER membrane. PMCID:PMC6367035*

Elucidation of the mechanism of ERAD (1996-now)

3. We are interested in how misfolded ER proteins are translocated back into the cytosol and degraded by the proteasome, a process called ERAD. One of our early contributions was the discovery that the p97 ATPase (Cdc48 in yeast) and its cofactors are required for the movement of poly-ubiquitinated proteins from the ER membrane into the cytosol. We showed that there are three distinct ERAD pathways (ERAD-L, -M, -C), depending on whether the misfolded domain is located in the lumen, membrane, or cytosol. We showed that the Hrd1 ubiquitin ligase is the crucial membrane component in ERAD-L, and that retro-translocation can be recapitulated with purified components. Based on a cryo-EM structure of the Hrd1 complex and molecular dynamics simulations, we established a new paradigm by which proteins cross membranes: translocation through a locally thinned membrane region. We also elucidated the mechanism of Cdc48 function, demonstrating that one ubiquitin molecule in the chain is unfolded and serves as initiator of substrate processing.

a. *Carvalho, P., Stanley, A.M., and Rapoport, T.A. (2010) Cell 143, 579-591. Retro-translocation of a misfolded luminal ER protein by the ubiquitin-ligase Hrd1p. PMCID: PMC3026631*
b. *Twomey, E.C., Ji, Z., Wales, T.E., Bodnar, N.O., Engen, J.R., and Rapoport, T.A. (2019) Science 365(6452). Substrate processing by the Cdc48 ATPase complex is initiated by ubiquitin folding. PMCID:PMC6980381*
c. *Wu, X., Mi, W., Siggel, M., Ovchinnikov, Mi, W., S., Svetlov, V., Nudler, E., Liao, M., Hummer, G., and Rapoport, T.A. (2020) Science 368(6489). Structural basis of ER-associated protein degradation mediated by the Hrd1 ubiquitin ligase complex. PMCID:PMC7380553*
d. *Li, H., Ji, Z., Paulo, J.A., Gygi, S.P., and Rapoport, T.A. (2024) Mol. Cell 84, 1290-1303. Bidirectional substrate shuttling between the 26S proteasome and the Cdc48 ATPase promotes protein degradation. PMID: 38401542*

Determining the mechanisms that shape the ER (2000-now)

4. How is the characteristic shape of an organelle generated and maintained? We started this new field by addressing how the morphology of the ER is generated. The ER is indeed intriguing, as it consists of a tubular network with interdispersed sheets. We have addressed how ER tubules are formed, how they are connected into a network, how sheets are formed, and how sheets are stacked on top of each other. We first used an in vitro assay to identify the reticulons as proteins required to shape ER tubules. Subsequent experiments showed that there is another protein family with a redundant function (REEPs/Yop1p). These proteins are both necessary and sufficient for tubule formation, probably by forming wedges in the membrane and arc-like scaffolds around the tubules. We then showed that ER membranes are fused by membrane-bound GTPases, atlastins in metazoans and RHD3- and Sey1p-related proteins in plants and yeast. These proteins interact across the fusing membranes and mediate homotypic fusion by undergoing a conformational change during GTP hydrolysis. We have clarified the fusion mechanism of atlastins, and showed that three-way tubular junctions are stabilized by the lunapark protein. Most recently, we have reconstituted an ER network with purified proteins. Sheets are also generated by the reticulons and DP1/Yop1p by stabilization of the high membrane curvature of sheet edges.

Finally, we showed that sheets are stacked by helical connections. Stacked ER sheets resemble a parking garage, where the different levels are connected by helical ramps.

a. *Voeltz, G.K., Prinz, W.A., Shibata, Y., Rist, J.M., and Rapoport, T.A. (2006) Cell 124, 573-86. A class of membrane proteins shaping the tubular endoplasmic reticulum.*
b. *Hu, J., Shibata, Y., Zhu, P.-P., Voss, C., Rismanchi, N., Prinz, W.A., Rapoport, T.A., and Blackstone, C. (2009) Cell 138, 549-561. A class of dynamin-like GTPases involved in the generation of the tubular ER network. PMCID: PMC2746359*
c. *Powers, R.E., Wang, S., Liu, T., and Rapoport, T.A. (2017) Nature 543, 257-260. Reconstitution of the tubular endoplasmic reticulum network with purified components. PMCID: PMC5853125*
d. *Wang, N., Shibata, Y., Paulo, J.A., Gygi, S.P., and Rapoport, T.A. (2023) Nat Commun 14, 4765. A conserved membrane curvature-generating protein is crucial for autophagosome formation in fission yeast. PMCID:PMC10409813*

Mechanism of protein import into peroxisomes (2019- now)

5. We became interested in the mechanism by which proteins are imported into peroxisomes. Surprisingly, these organelles can import folded and even oligomeric proteins. This is different from translocation into the ER or mitochondria, where proteins are unfolded during membrane passage. We recently solved the mystery by demonstrating that peroxisomal protein import resembles nuclear transport. The membrane protein PEX13 has a tyrosine/glycine-rich YG domain, which forms a selective phase, similar to the FG phase formed by nucleoporins in nuclear pores. The receptor PEX5 partitions into the phase, using aromatic motifs in its unstructured N-terminus, and brings cargo along. The complex of PEX5 and cargo moves all the way into peroxisomes. Then, PEX5 returns through a separate retro-translocon. A cryo-EM structure showed that the channel is formed from a heterotrimeric ubiquitin ligase complex. During retro-translocation, the receptor is unfolded, releasing cargo inside the lumen.

a. Feng, P., Wu, X., Erramilli, S.K., Paulo*, J.A., Knejski, P., Gygi, S.P., Kossiakoff, A.A., and Rapoport, T.A. (2022), Nature 607, 374-380. A peroxisomal ubiquitin ligase complex forms a retrotranslocation channel. PMCID:PMC9279156*
b. *Skowyra, M.L. and Rapoport, T.A. (2022), Mol. Cell 82(17), 3209-25. PEX5 translocation into and out of peroxisomes drives matrix protein import. PMCID:PMC9444985*
c. *Gao, Y., Skowyra, M.L., Feng, P. and Rapoport, T.A. (2022), Science 378(6625), eadf3971. Protein import into peroxisomes occurs through a nuclear pore-like phase. PMCID:PMC9795577*

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/12e_sq65Vx556/bibliography/40925740/public/?sort=date&direction=descending

BIOGRAPHICAL SKETCH

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NAME: Rudolf Pisa

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

POSITION TITLE: Postdoctoral Fellow

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 Cambridge (Cambridge, UK)	B.A.	06/2012	Chemistry
University of Cambridge (Cambridge, UK)	M.Sci.	06/2012	Chemistry
Rockefeller University (New York, NY, USA)	Ph.D.	10/2019	Biochemistry
Harvard Medical School (Boston, MA, USA)	Post-Doc	present	Cell Biology

A. Personal Statement

I am continually inspired by the complexity of molecular interactions that underlie biology. Throughout my research career, I have aimed to decipher the molecular and mechanistic principles that drive dynamic cellular processes such as cell division and protein synthesis and degradation. To tackle these research problems, I have learned and applied cutting-edge interdisciplinary approaches in chemistry, biochemistry, molecular biology, and structural biology. My passion for research that interfaces chemistry and biology originated during my undergraduate training with Dr. Jason Chin in Cambridge, UK where I synthesized unnatural amino acids endowed with reactive functional groups. Through this work I acquired critical skills in chemical synthesis and learned many molecular biology techniques.

To continue expanding my skillset, I joined Dr. Tarun Kapoor's laboratory at Rockefeller University in New York for my graduate training. In Dr. Kapoor's lab, I pioneered a novel approach to design chemical inhibitors – Resistance Analysis During Design – "RADD". This method principally relies on engineering mutations in the inhibitor binding site to map interactions between the inhibitor and its target protein. Identifying these interactions is crucial for optimizing inhibitor potency and selectivity. Not only did this work allow me to develop new skills in biochemistry and structural biology, but it also led to the design of spastazoline, the first cell permeable chemical probe for the microtubule-severing protein spastin.

As I advanced in my research endeavors, I began to seek out mentoring opportunities through the undergraduate research program at Rockefeller, which aims to train the next generation of young scientists. It was particularly gratifying to design projects and mentor two talented undergraduate students who excelled in the lab, and both successfully applied to graduate schools. One of these projects, which focused on identifying resistance mechanisms to an inhibitor of kinesin Cenp-E, led to a publication (2) along with my thesis work.

For my postdoctoral training, I wanted to combine my interdisciplinary skills to address outstanding questions in cell biology, and I joined Dr Tom Rapoport's laboratory to study the biochemical principles of endoplasmic reticulum-associated protein degradation (ERAD). ERAD pathways are essential for maintaining protein homeostasis in cells and play a role in many diseases in which mutant proteins are misfolded, as well as in the degradation of endogenous proteins. To study ERAD, I employ approaches in yeast genetics and membrane protein biochemistry with the aim to elucidate how substrates are recognized and processed by the ERAD machinery. My ultimate goal is to reconstitute the machinery with purified components and to visualize it by cryo-electron microscopy.

To support my training in Dr Rapoport's lab, I secured funding from the American Heart Association, Charles A King Trust, and K99/R00 from NIGMS/NIH. As part of my fellowships, I received important training in science communication (both spoken and written), mentorship, and research ethics. I mentored a student last summer that allowed me to build on my experience from grad school in teaching, motivating and project planning. My

ultimate goal is to become an independent investigator at an academic institution. I plan to develop a research program that combines my strong chemical biology expertise with the new experimental skills I am acquiring in postdoctoral training in order to study molecular mechanisms of protein homeostasis and degradation in cells.

1. **Pisa R** and Kapoor TM. Chemical strategies to overcome resistance against targeted anticancer therapeutics. *Nat Chem Biol, 2020, 16, 8, 817-825.* PMCID: PMC7510053
2. **Pisa R**, Phua DYZ and Kapoor TM. Distinct mechanisms of resistance to a CENPE inhibitor emerge in haploid and diploid cells. *Cell Chem Biol, 2020, 27, 7, 850-857.* PMCID: PMC7444662
3. **Pisa R**, Cupido T, Steinman JB, Jones NH, Kapoor TM. Analyzing resistance to design selective chemical inhibitors for AAA proteins. *Cell Chem Biol, 2019, 26, 9, 1-11*. PMCID: PMC6754270 (Art depicting work in this manuscript was selected for journal cover).
4. **Pisa R***, Cupido T*, Kapoor TM. Designing allele-specific inhibitors of spastin, a microtubule-severing AAA protein. *JACS, 2019, 141, 14, 5602-5606. *equal contributions.* PMCID: PMC6637947

B. Positions, Scientific Appointments, and Honors

Positions and Employment:

2020-present	**Postdoctoral Fellow**, Tom A Rapoport's laboratory, Department of Cell Biology, Harvard Medical School, Boston, US
2013- 2019	**Graduate Fellow**, Dr Tarun M Kapoor's laboratory, Rockefeller University, New York, US
2012- 2013	**Graduate Student**, ~3-month rotations in labs across Tri-Institutional PhD Program in Chemical Biology, Rockefeller University, New York, US
2011- 2012	**Undergraduate Researcher**, Dr Jason W Chin's laboratory, Department of Chemistry and MRC Laboratory of Molecular Biology, University of Cambridge, UK

Honors:

9/2024-current	NIGMS/NIH K99/R00 award (K99GM154114)
7/2024	Poster Award, GRC Protein Secretion and Trafficking, NH, US
2023-2025	King Trust Postdoctoral Research Fellowship (relinquished 8/2024)
2021-2023	American Heart Association Postdoctoral Fellowship
9/2019	Poster prize (1st place), TPCB Chemical Biology Symposium, Rockefeller University, NY
8/2019	HHMI Travel Award - Junior Scientist Workshop on Solving Biological Problems with Chemistry, HHMI Janelia Campus, Ashburn, Virginia, US
9/2017	Poster prize (1st place), TPCB Chemical Biology Symposium, MSKCC, New York, US
7/2014	EMBO Travel Award, Molecular genetics with fission yeast, Institute Pasteur, Paris, France
2010-2011	Baden–Württenberg Stipendium, Cambridge-Heidelberg Exchange Programme, Germany
2009	Skerne (1745) Scholarship, First Class Honors, St Catharine's College, Cambridge, UK
2007-2012	Cambridge European Trust Scholarship, University of Cambridge, UK

Professional Societies:

2020-present	Member, Harvard Medical Postdoctoral Association
2020-2023	Member, American Heart Association
2017-2018	Mentor, Summer Undergraduate Research Fellow (SURF) Program, Rockefeller University
2014-2018	Member, Student Organizing Committee, TPCB Interview Open Day, Rockefeller University
2014	Chair, Organizing Committee, TPCB Chemical Biology Symposium, Rockefeller University
2008-2020	Member, Royal Society of Chemistry, UK

C. Contributions to Science

1. **Graduate Training:** My graduate work in Dr Tarun Kapoor's lab at Rockefeller University was centered on the design of chemical inhibitors and on characterizing their physiologic targets in cells. My main project focused on the design of inhibitors for proteins in the AAA (ATPases Associated with diverse

cellular Activities) superfamily, for which only a handful of chemical probes existed, and no inhibitor-protein binding models were available. I chose to work on the AAA protein spastin, which can sever microtubule filaments in vitro, and which is needed in cells for successful cell division and organelle transport. I purified wild-type spastin and several spastin alleles with mutations in the ATP-binding site that do not disrupt biochemical activity. I then tested these proteins against compounds that bind in the ATP pocket. These data revealed specific residue-inhibitor contacts that guided development of computational models we needed for optimization of the compound potency and selectivity. We named this approach RADD (Resistance Analysis During Design) and used it to develop spastazoline, the first cell-permeable, potent and selective chemical probe for spastin. This approach also revealed mutations in spastin that confer resistance to spastazoline in biochemical and cellular assays. I leveraged these RADD-predicted mutations to show that spastazoline interacts with spastin in living cells. Further, I solved X-ray co-crystal structures of inhibitor-bound spastin complexes that matched our RADD computational models. In a follow-up project, I used these models to design spastazoline analogs that can overcome resistance to spastazoline. These studies establish RADD as a useful method for how chemical inhibitors can be designed and provided new chemical biology tools to study spastin in vitro and in cells. Investigating spastin's roles in cell division using tools I generated remains an active research area in the Kapoor lab. My graduate work led to 7 manuscripts in peer-reviewed journals (4 as first author), one book chapter, one patent and several presentations at national and international conferences.

Patent:

a. Kapoor TM, Cupido T, **Pisa R**, *"2,4-diaminopyrimidine bicycles for treating cancer".* Patent Application: PCT/US2019/064937. Publication number: WO2020118183A1

Book Chapter:

b. **Pisa R**, Cupido T, Kapoor TM. *Chemical approaches to analyze biological mechanisms and overcome resistance to therapeutics.* Book chapter in "Advanced Chemical Biology: Chemical Dissection and Reprogramming of Biological Systems", Wiley, 2023, ISBN: 9783527347339.

Presentations:

c. *Analyzing resistance to design selective chemical inhibitors for AAA proteins.* Talk and poster presentation, Junior Scientist Workshop on Solving Biological Problems with Chemistry, HHMI Janelia Research Campus, July 28-Aug 1, 2019, Ashburn, Virginia.
d. *Rationally designing potent and selective chemical inhibitors of AAA+ proteins*. Poster, Alpine Winter Conference on Medicinal and Synthetic Chemistry, February 2018, St Anton, Austria.

2. **Postdoctoral career:** The goal of my post-doctoral research in Dr Tom Rapoport's laboratory is to elucidate how cells recognize misfolded proteins in the endoplasmic reticulum (ER) and how are these substrates moved across the ER membrane into the cytosol for degradation - process known as ERAD (ER-associated degradation). Dr Rapoport has a reputation for performing outstanding high-impact research in membrane protein biochemistry and structural biology that led to seminal contributions in the fields of protein translocation across membranes as well as ERAD. Under Dr Rapoport's guidance and mentorship, I am building upon my graduate training in chemical biology and expanding my skills in reconstitution biochemistry and structural biology. To this end I used disulfide crosslinking to covalently trap ERAD substrates as they traverse the ER membrane. This work led to my first publication during postdoctoral training and provided a platform for future analysis of ERAD complexes using structural biology techniques, such as single particle cryogenic electron microscopy (cryo-EM). The goal for the rest of my postdoc is to gain expertise in cryo-EM and use this technique to elucidate how membrane-embedded ubiquitin ligases handle substrates. This will help answer major outstanding questions in the ERAD field and lay the groundwork for work on protein homeostasis in cellular organelles I plan to pursue in my future independent laboratory.

Manuscript:

a. **Pisa R** and Rapoport TA. Disulfide-crosslink analysis of the ubiquitin ligase Hrd1 complex during endoplasmic reticulum-associated protein degradation. *JBC, 2022, 298, 9, 102373.* PMCID: PMC9478403

Presentations:

b. 3/2024, Ubiquitin and Friends Symposium, Vienna, Austria. Presented a talk selected from abstracts: *Through thick and thin: How a thin membrane drives substrate selection during ER-Associated Degradation.*
c. 7/2024, Gordon Research Conference – Protein processing, Trafficking and Secretion. Presented a poster: *A thin membrane drives substrate selection during ER-associated degradation. (poster prize)*

3. **Peer Review:** Under guidance of my graduate and postdoctoral mentors, I have contributed to peer review of over 15 manuscripts in various journals. I served as a reviewer in study sections for the American Heart Association pre- and post-doctoral fellowships in Basic sciences (Membrane and Subcellular Organelles, 2022-23 cycle). This work involved triage of submitted applications, providing critiques, and participating in a Peer Review meeting. Since 2022 I volunteer to review manuscripts for STAR Protocols.

Complete List of Published Work:
Peer-reviewed articles: 8 (5 as first author); Non-peer reviewed (Book Chapter; Patent): 2 (1 as first author).

My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/165-PU92nnaks/bibliography/public/

BIOGRAPHICAL SKETCH

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NAME: Anna-Katharina Pfitzner

eRA COMMONS USER NAME (credential, e.g., agency login): ORCID 0000-0001-9952-5525

POSITION TITLE: Postdoctoral fellow

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
Eberhardt Karls University Tuebingen, Germany	BSc	07/2014	Biochemistry
Eberhardt Karls University Tuebingen, Germany	MSc	09/2016	Biochemistry
University of Geneva, Switzerland	PhD	10/2021	Life Science, Section: Molecular Biology and Physics of Life
Harvard Medical School, Boston, MA	Postdoctoral Fellow	present	Cell Biology and Structural Biology

A. Personal Statement

My main research interests are focused on understanding the molecular interaction of proteins with lipid membranes and their contribution to functionality for cells. Following these interests, I started my research career in the laboratory of Dr. Doron Rapaport studying the insertion of membrane proteins in mitochondria (1). Later I pursued these interests further in my graduate work on protein-driven membrane deformation and fission in the group of Aurelien Roux (2). Originally having undergone academic training in biochemistry and yeast cell biology, I expanded my skillset towards biophysical techniques and approaches during my graduate work. After joining the laboratory of Dr. Tom Rapoport for my postdoctoral research which aims to unravel the molecular details of transmembrane protein extraction from the ER-membrane in mammalian cells, I further complemented my experimental techniques with training in cryogenic electron microscopy and mammalian cell biology. Taking advantage of my diverse training background, I hope to establish my own original research direction in the field of membrane biology as an independent researcher in the future. I envision using diverse techniques and approaches such as applying membrane biophysics to tackle gaps and open questions in cell biological problems.

1. **Pfitzner, A.K.,** Steblau, N., Ulrich, T., Oberhettinger, P., Autenrieth, I.B., Schütz, M., Rapaport, D. (2016) Mitochondrial-bacterial hybrids of BamA/Tob55 suggest variable requirements for the membrane integration of β-barrel proteins. ***Sci Rep.*** 6:39053.
2. **Pfitzner, A.K.,** Mercier, V., Jiang, X., Moser von Filseck, J., Baum, B., Šarić, A., Roux, A. (2020). An ESCRT-III polymerization sequence drives membrane deformation and fission. ***Cell.*** 182(5):1140-1155.e18.

B. Positions and Scientific Appointments,

2022 - present **Research Fellow**, Harvard Medical School, Prof. Tom Rapoport
2017 - 2021 **PhD Candidate**, Graduate School of Life Sciences, University of Geneva, Prof. Aurelien Roux

2015 - 2016 **Undergraduate Research Associate** and Master thesis work, University of Tuebingen, Lab Prof. Doron Rapaport
2014 **Undergraduate Intern**, Pennsylvania State University, US, Lab Prof. David Boehr
2014 **Bachelor thesis work**, University of Tuebingen, Prof Stefan Stefanovic
2013 **Undergraduate Intern**, University of Edinburgh, UK, Lab Dr. Laura Spangolo

Honors

2023 - present **HFSP** long-term postdoctoral Fellowship
2022 - 2024 **EMBO** postdoctoral Fellowship
2022 **Prix Schlaeffli 2022**, Swiss Academy of Science, **best PhD** thesis in Biology in **Switzerland**
2022 **Laemmli Prize 2022** for **exceptional thesis** in Molecular Biology at **University of Geneva**
2020 **Birnstiel Award 2020**, IST Austria, for **outstanding PhD thesis** in Life Sciences (**International competition**)
2014 Scholarship of the DAAD (German Academic Exchange Service) rise worldwide program
2013 Scholarship of the DAAD (German Academic Exchange Service) rise worldwide program

C. Contributions to Science

1. Discovery and characterization of an ATP fueled ESCRT-III recruitment sequence driving ESCRT-III catalyzed membrane deformation and fission

ESCRT-III proteins polymerize into multi-subunit filaments which drive membrane deformation and fission during many crucial cellular processes like cytokinesis and nuclear envelope closure by an unknown mechanism. Using diverse in vitro reconstitution assays combined with electron microscopy experiments, we observed dynamic changes in the subunit composition of ESCRT-III filaments over time driven by the ESCRT-III associated AAATPase Vps4. We further were able to link the altering subunit composition to a gradual change in filament architecture from a flat spiral to an intermediate conical spiral and finally a constricted helix which was able to drive membrane fission in vitro. Overall, we propose a new mechanistical model and novel concept on how ESCRT-III polymers can drive both, membrane deformation and fission. Physical feasibility of the proposed models was thoroughly tested and insured using molecular simulation approaches in a collaborative effort.

i) **Pfitzner, A.K.,** Mercier, V., Jiang, X., Moser von Filseck, J., Baum, B., Šarić, A., Roux, A. (2020). An ESCRT-III polymerization sequence drives membrane deformation and fission. ***Cell.*** 182(5):1140-1155.e18.
ii) Meadowcroft, B., Palaia, I., **Pfitzner, A.K.**, Roux, A., Baum, B., Šarić, A. (2022) Mechanochemical Rules for Shape-Shifting Filaments that Remodel Membranes. ***Phys Rev Lett***. 129(26):268101.
iii) Jiang, X., Harker-Kirschneck, L., Vanhille-Campos, C., **Pfitzner, A.K.**, Lominadze, E., Roux, A., Baum, B., Šarić, A. (2022) Modelling membrane reshaping by staged polymerization of ESCRT-III filaments. ***PLoS Comput Biol***. 18(10):e1010586.
iv) Buysse, D., **Pfitzner, A.K.**, West, M., Roux, A., Odorizzi, G. (2020). The ubiquitin hydrolase Doa4 directly binds Snf7 to inhibit recruitment of ESCRT-III remodeling factors in *S. cerevisiae*. ***J Cell Sci*.** 28;133(8):jcs241455.
v) **Pfitzner, A.K.,** Moser von Filseck, J., Roux, A. (2021). Principles of membrane remodelling by dynamic ESCRT-III polymers. ***Trends Cell Biol.*** 31(10):856-868.

2. Description and functional characterization of novel ESCRT-III subcomplex and membrane bound polymer (supervision of Master project of H. Zivkovic**)**

Before this work, Vps60 was a poorly characterized member of the ESCRT-III family of proteins. Using diverse biochemical and biophysical techniques we systematically analyzed membrane binding, polymerization and ESCRT-III subunit interaction properties of Vps60 in vitro, arriving at the conclusion that Vps60 behaves as an early acting subunit with the capability of initiating an alternative ESCRT-III polymer. The project was started as a Master thesis project under my supervision and later concluded by me.

i) **Pfitzner, A.K.*,#**, Zivkovic, H.*, Bernat-Silvestre, C., West, M., Peltier, T., Humbert, F., Odorizzi, G., Roux, A.# (2023) Vps60 initiates alternative ESCRT-III filaments. ***J Cell Biol***. 222(11):e202206028.

* co-first authors
# co-correspondence authors

3. Biophysical studies of Mgm1 polymerization on different membrane geometry

A member of Dynamin superfamily, Mgml (OPAl in mammals) is required for cristae organization and fusion of the IM during mitochondrial fusion. How Mgml performs membrane fusion and acts in cristae organization is elusive. In this publication, the crystal structure of Mgml from the fungus *Chaetomium thermophilus* was determined and domain functions were assigned by functional *in vivo* and *in vitro* analysis of mutants designed based on the solved structure. Contributing dynamic biophysical measurements and analysis to the study, I quantified Mgml binding to curved membrane templates revealing the proteins strong preference for filament nucleation in highly curved template regions. This is in line with Mgml 's functions in cristae organization which depict a similar high curvature membrane architecture. Force exertion on a membrane nanotube upon GTP hydrolysis was further indicative of a collective behavior of the Mgml polymers, likely by structural rearrangements driving a GTP-dependent expansion or constriction of positively or negatively curved membrane tubes, respectively. In conclusion, the study shows Mgml to polymerize in a left-handed helical filament on or in tubular membrane structures, which likely undergo membrane remodeling by GTP dependent filament rearrangement. The study proposes, that Mgml polymers, due to their left-handed assembly, would expand positively curved membrane structures and constrict negatively curved membrane structures by dynamin-like power stroke movements, explaining how Mgml acts in fusion of the mitochondrial IM.

i) Faelber, K.*, Dietrich, L.*, Noel, J., Wollweber, F., **Pfitzner, A.K.**, Mühleip, A., Sánchez, R., Kudryashev, M., Chiaruttini, N., Lilie, H., Schlegel, J., Rosenbaum, E., Hessenberger, M., Matthaeus, C., Noé, F., Roux, A., van der Laan, M., Kühlbrandt, W., Daumke, O. (2019) Structure and assembly of the mitochondrial membrane remodeling GTPase Mgm1. ***Nature.*** 571(7765):429-433.

* co-first authors

4. Functional study and analysis of conservation in biogenesis of β-barrel membrane proteins in mitochondria and gram-negative bacteria focusing on POTRA domains in Tob55/BamA and inter membrane space chaperones (Student Research Associate and Master thesis work**)**

Insertion of beta barrel into the outer membranes of mitochondria and gram-negative bacteria is highly conserved demonstrated by the ability of bacterial proteins to be correctly inserted into mitochondria and *visa versa*. In this study we exploit the conservation between both systems to address the specific function of POTRA domains, an inter membrane space domain with varying copy numbers across bacteria and mitochondria, during insertion of bacterial and mitochondrial proteins using yeast and bacterial cell biology.

i) **Pfitzner, A.K.,** Steblau, N., Ulrich, T., Oberhettinger, P., Autenrieth, I.B., Schütz, M., Rapaport, D. (2016) Mitochondrial-bacterial hybrids of BamA/Tob55 suggest variable requirements for the membrane integration of β-barrel proteins. ***Sci Rep.*** 6:39053.

5. Peer Review

I provided peer reviews of manuscripts for the journals Current Genetics and Journal of Cell Science. Further, I have contributed to peer review of over 10 manuscripts under my graduate and postdoc supervisors for, amongst others, journals like Nature Cell Biology and Science.

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: Li, Hao

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

POSITION TITLE: Postdoctoral Fellow

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
University of Science and Technology of China, Hefei, Anhui	BS	06/2014	Chemistry
University of Wisconsin-Madison, Madison, WI	PHD	07/2020	Pharmaceutical Sciences
Harvard Medical School, Boston, MA	Postdoctoral Fellow	present	Biochemistry

A. Personal Statement

My academic training and research experience in organic chemistry, chemical biology, structural biology, and biochemistry provided me with multidisciplinary background for my development into an independent researcher. My research career started with organic synthesis methodology in the catalytic alkylation of primary allylic amines, under the supervision of Dr. Shi-Kai Tian. After I joined the lab of Dr. Jiaoyang Jiang as a graduate student, my research directions transitioned to chemical biology and structure biology. I developed novel covalent probes for O-GlcNAc Transferase and determined the structures of human O-GlcNAcase in complex with its substrates, for the first time. My graduate research yielded two co-first authored papers in prestigious journals and several awards. I joined Dr. Tom Rapoport's lab to pursue my postdoctoral research in structural biology, biochemistry, and cell biology, where he is a renowned leader in the field. My initial work during my postdoctoral research addressed the mechanism by which Cdc48 processes its substrates, resulting in a co-first authored paper. I then used purified yeast components to reconstitute Cdc48-dependent degradation of well-folded model substrates by the 26S proteasome. Based on this in vitro reconstitution assay, I found that minimal system consists of the 26S proteasome, the Cdc48-UN ATPase complex, the proteasome cofactor Rad23, and the Cdc48 cofactors Ubx5 and Shp1. I plan to expand my skillsets by answering the questions of 1) which additional factors are required for Cdc48/p97-dependent proteasomal protein degradation and 2) what is the molecular mechanism of Cdc48 cofactors mediated substrate recruitment and processing. My proposed research under the supervision of Dr. Rapoport will extend my expertise in biochemical experiments and allow me to acquire proficiency in cryogenic electron microscopy and cell culture. The proposed project also includes a detailed career development plan through which my skills in scientific writing and communication, as well as in mentoring students and lab management will be elevated. Taken together, I believe that the proposed research and training plan will pave my road to become an independent investigator in the fields of biochemistry and chemical biology.

1. **Li H**, Ji Z, Paulo J, Gygi S, Rapoport T. Bidirectional substrate shuttling between the 26S proteasome and the Cdc48 ATPase promotes protein degradation. BioRxiv. 2023 December; 2023.12.20.572403. DOI: 10.1101/2023.12.20.572403. PMCID: PMC10769200

2. Ji Z, **Li H**, Peterle D, Paulo J, Ficarro S, Wales T, Marto J, Gygi S, Engen J, Rapoport T. Translocation of polyubiquitinated protein substrates by the hexameric Cdc48 ATPase. *Molecular Cell*. 2022 February; 82(3):570-584.e8. DOI: 10.1016/j.molcel.2021.11.033. PMCID: PMC8818041 (**Co-first Author**)
3. Hu C, Worth M, Fan D, Li B, **Li H**, Lu L, Zhong X, Lin Z, Wei L, Ge Y, Li L, Jiang J. Electrophilic probes for deciphering substrate recognition by O-GlcNAc transferase. *Nature Chemical Biology*.

2017 October 23; 13(12):1267-1273. DOI: 10.1038/nchembio.2494. PMCID: PMC5698155 (**Co-first Author**)

4. Li B, **Li H**, Hu C, Jiang J. Structural insights into the substrate binding adaptability and specificity of human O-GlcNAcase. *Nature Communications*. 2017 September 22; 8(1). DOI: 10.1038/s41467-017-00865-1. PMCID: PMC5610315. (**Co-first Author**)

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2023 -	Member, The American Society for Cell Biology
2020 -	Research Fellow, Harvard Medical School
2017 - 2020	Research Assistant, University of Wisconsin-Madison, Madison, WI
2015 - 2015	Graduate Fellowship, Wisconsin Distinguished Graduate Fellowship - Thora Vervoren Fellowship
2014 - 2017	Teaching Assistant, University of Wisconsin-Madison, Madison, WI
2013 - 2013	Exchange student, University of Oxford, Oxford

Honors

2024 - 2024	Robin Reed Memorial Travel Award, Harvard Medical School
2019 - 2019	Witiak (Drug Discovery) Graduate Student Travel Award, University of Wisconsin-Madison
2019 - 2019	Dr. Razia Zaman-Dr. Shahanara Zaman Saroya Graduate Award for Excellence in Research & Scholarship, University of Wisconsin-Madison
2016 - 2017	School of Pharmacy Teaching Assistant 2016-17 performance award, University of Wisconsin-Madison
2012 - 2013	Outstanding Undergraduate Scholarship, University of Science and Technology of China
2011 - 2012	Xuxin Scholarship, University of Science and Technology of China
2010 - 2011	Outstanding Undergraduate Scholarship, University of Science and Technology of China

C. Contribution to Science

1. **Determine the structure of human O-GlcNAcase and reveal its substrate binding mode.** O-GlcNAcase (OGA) specifically hydrolyzes the post-translational modification O-GlcNAcylation, and its dysregulation is linked to Alzheimer's Disease. Before my work, only the structure of bacterial OGA has been determined, but it lacked important domains of the human homolog and cannot provide information about how the human protein bind to substrates. Together with a colleague, I determined the crystal of human OGA in its apo form, and in complex with a few glycopeptide substrates, for the first time. Moreover, we found that these glycopeptides bind in a bidirectional yet conserved conformation within the substrate-binding cleft of OGA. This study provides fundamental insights into a general principle that confers the substrate binding adaptability and specificity to OGA in O-GlcNAc regulation.

    a. Li B, **Li H**, Hu C, Jiang J. Structural insights into the substrate binding adaptability and specificity of human O-GlcNAcase. *Nature Communications*. 2017 September 22; 8(1). PMCID: PMC5610315 (**Co-first Author**)
    b. Li B, **Li H**, Lu L, Jiang J. Structures of human O-GlcNAcase and its complexes reveal a new substrate recognition mode. *Nature Structural & Molecular Biology*. 2017; 24(4):362-369. PMCID: PMC8171356

2. **Develop covalent probes and covalent inhibitors of O-GlcNAc Transferase.** O-GlcNAc Transferase (OGT) is an essential human glycosyltransferase that adds O-GlcNAc modifications to numerous proteins. However, little is known about the mechanism with which OGT recognizes various protein substrates. Together with colleagues, I designed and synthesized electrophilic probes that can covalently link OGT with its substrates. The probe was used to report the impacts of OGT mutations on protein substrate or sugar binding and to discover OGT residues crucial for protein recognition. Based on the probes, I then synthesized and a panel of covalent inhibitors of OGT with high potency and specificity. The inhibitors suppressed growth of tumor cells.

a. Hu C, Worth M, Fan D, Li B, **Li H**, Lu L, Zhong X, Lin Z, Wei L, Ge Y, Li L, Jiang J. Electrophilic probes for deciphering substrate recognition by O-GlcNAc transferase. *Nature Chemical Biology*. 2017 October 23; 13(12):1267-1273. PMCID: PMC5698155 (**Co-first Author**)
b. Worth M, Hu C, **Li H**, Fan D, Estevez A, Zhu D, Wang A, Jiang J. Targeted covalent inhibition of O-GlcNAc transferase in cells. *Chemical Communications*. 2019; 55(88):13291-13294. PMCID: PMC6823131

3. **Reveal the mechanisms by which the Cdc48 ATPase translocates polyubiquitinated protein substrates and mediates their degradation by the proteasome.** The AAA ATPase Cdc48 is responsible for extraction of proteins from their binding partners and preparing them for degradation by the proteasome, but its molecular mechanism remains unclear. We found that polyubiquitinated substrate binds to the ATPase complex through the Ufd1/Npl4 (UN) cofactor. One of the ubiquitin molecules (initiator ubiquitin) is unfolded and projects its N-terminus across both ATPase rings. ATP hydrolysis by the D2 ring ATPases moves the ubiquitin molecules linked to the C-terminus of the initiator (proximal ubiquitin molecules, in pink) and ultimately the substrate through the central pore and causes their unfolding. I further developed an in vitro degradation system with purified proteins. Using this system, I found that substrates are shuttled between Cdc48 ATPase and the proteasome before being degraded. These findings greatly deepened our understanding of the molecular mechanism of Cdc48 and its role in protein degradation pathway.

    a. **Li H**, Ji Z, Paulo J, Gygi S, Rapoport T. Bidirectional substrate shuttling between the 26S proteasome and the Cdc48 ATPase promotes protein degradation. BioRxiv. 2023 December; 2023.12.20.572403. DOI: 10.1101/2023.12.20.572403. PMCID: PMC10769200
    b. Ji Z, **Li H**, Peterle D, Paulo J, Ficarro S, Wales T, Marto J, Gygi S, Engen J, Rapoport T. Translocation of polyubiquitinated protein substrates by the hexameric Cdc48 ATPase. *Molecular Cell*. 2022 February; 82(3):570-584.e8. PMCID: PMC8818041 (**Co-first Author**)