Structural basis of membrane protein chaperoning through the mitochondrial intermembrane dpace

K. Weinhäupl[1], C. Lindau[2,3], A. Hessel[1] Y. Wang[4] C. Schütze[2], T. Jores,[5], H. Kalbacher[5], D. Rapaport[5], M. Brennich[6], K. Lindorff-Larsen[4], N. Wiedemann[2,6], P.l Schanda[1]

[1]Institut de Biologie Structurale, CEA, CNRS, Université Grenoble Alpes, 38042 Grenoble, France, [2]Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine, University of Freiburg, 79104 Freiburg, Germany, [3]Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany, [4]Structural Biology and NMR Laboratory, The Linderstrøm-Lang Centre for Protein Science, Department of Biology, University of Copenhagen, DK-2200 Copenhagen, Denmark, [5]Interfaculty Institute of Biochemistry, University of Tübingen, 72076 Tübingen, Germany, [6]European Molecular Biology Laboratory, 38042 Grenoble, France, [7]BIOSS Centre for Biological Signalling Studies, University of Freiburg, 79104 Freiburg, Germany, **katharina.weinhaupl@ibs.fr**

The diverse group of molecular chaperones is dedicated to accompany, fold and protect other proteins until they reach their final conformation and location inside the cell. To this end, molecular chaperones need to be specialised in performing specific tasks, like folding, transport or disaggregation, and versatile in their recognition pattern to engage many different client proteins. Moreover, molecular chaperones need to be able to interact with each other and with other components of the protein quality control system in a complex network. Interactions between the different partners in this network and between the substrate and the chaperone are often dynamic processes, which are especially difficult to study using standard structural biology techniques. Consequently, structural data on chaperone/substrate complexes are sparse, and the mechanisms of chaperone action are poorly understood. In this work I present investigations on the structure, dynamics and substrate interactions of the mitochondrial intermembrane space chaperone TIM910, using various biophysical and *in vivo* methods.

TIM910 binds its client membrane proteins in a highly dynamic manner. Not only is the TIM910 complex in constant exchange between monomeric and hexameric species, but also the bound substrate samples multiple conformations on a millisecond timescale. Based on nuclear magnetic resonance (NMR), small-angle X-ray scattering (SAXS), analytical ultracentrifugation (AUC) and *in vivo* mutational experiments I propose a structural model of the chaperone/membrane protein interaction. TIM910 binds its substrates in a hydrophobic pocket on the exterior of the chaperone in a modular fashion, where the number of TIM910 complexes bound depends on the length of the substrate. Moreover, TIM910 uses the same binding site to transport client proteins that differ significantly in their membrane inserted conformation.

Although the X-ray structure of apo TIM910 has been solved more than ten years ago [1], it provided no evidence on where or how substrates are bound. The case of TIM910 shows that static structural data is often not enough to explain how a molecular system works, and only the combination of different techniques, allowed us to understand this chaperone/substrate complex at the atomic level.

References

[1] - C.T. Webb, M.A. Gorman, M. Lazarou, M.T. Ryan and J.M. Gulbis, Crystal structure of the mitochondrial chaperone TIM9.10 reveals a six- bladed alpha-propeller. *Molecular cell 21* (2006), 123-33.