Mitochondria are organelles of eukaryotic cells, which are required for several essential cellular processes including energy production, biosynthesis of iron-sulfur cluster and heme, metabolism of amino acids and lipids, signaling, and apoptosis. Mitochondrial dysfunctions have been implicated in neurodegenerative illnesses like Parkinson's and Alzheimer's disease, in the development of cancer and in aging. To properly fulfill their role in the cell, mitochondria harbor a diverse set of proteins most of which are encoded by nuclear DNA. These proteins are translated on cytosolic ribosomes and imported into the mitochondria by dedicated machineries.

To date, we have a rather detailed knowledge of how precursor proteins are imported into the matrix, the innermost compartment of mitochondria. In contrast to this, the biogenesis of mitochondrial outer membrane proteins is less well understood. The aim of my work is to shed new light on how proteins are targeted to and inserted into the mitochondrial outer membrane. For this, I focused on two distinct classes of mitochondrial outer membrane proteins, namely multispan proteins, which are inserted into the membrane via two or more α-helical transmembrane domains, and β-barrel proteins, which span the membrane with multiple β-strands that together form a cylindrical structure.

Unlike the majority of mitochondrial protein, multispan outer membrane proteins do not require the transmembrane pore formed by the translocase of the outer membrane (TOM) complex for their biogenesis. Their import was, however, shown to be dependent on two outer membrane proteins, Tom70 and Mim1. In our efforts to better understand the biogenesis of multispan proteins, we performed a high throughput screen to find additional proteins that might be involved in this process. In this screen, we identified the cardiolipin synthase Crd1 as a potential candidate. In follow-up experiments, I could show that a deletion of the *CRD1* gene does indeed hamper the biogenesis of mitochondrial outer membrane multispan proteins. A deletion of *GEP4*, a gene encoding for another protein in the biosynthesis pathway of the mitochondria-specific lipid cardiolipin, leads to a similar phenotype. Collectively, our findings show that the biogenesis of mitochondrial outer membrane multispan proteins is not only dependent on proteins in the outer membrane, but also on the mitochondria-specific phospholipid cardiolipin.

For β-barrel proteins, we have a rather detailed understanding of the import path these proteins take once they reach the mitochondrial surface. Here, they interact with the TOM complex that translocates them into the intermembrane space from where they are inserted into the outer membrane with the help of the TOB complex. When I started my work, however, it was unclear how the β-barrel proteins, after their synthesis in the cytosol, are specifically targeted to the mitochondria. In my study, I could identify a β-hairpin motif as the minimal signal that ensures the correct targeting of mitochondrial β-barrel proteins. Such a β-hairpin motif is composed of two β-strands that are connected by a short loop. I could further show, that the β-sheet of this targeting signal has one highly hydrophobic face and that the hydrophobicity is crucial for the targeting capacity of the motif. Finally, I could demonstrate that a peptide composed of a β-targeting signal can specifically interact with the mitochondrial import receptor Tom20. Taken together, I could identify the signal that targets β-barrel proteins to mitochondria and helps to initiate import by interacting with Tom20.