Mitochondria are cellular compartments that are very well known for their roles in metabolism and energy conversion. The set of proteins building mitochondria and responsible for their function, called the proteome, originates from two different genomes. The vast majority, 99% of these proteins are encoded by the nuclear genome and about 1% is encoded by the mitochondrial genome. The proteins encoded by the nuclear genes and synthesized on the cytosolic ribosomes must be properly and efficiently imported into the mitochondria. The import of mitochondrial proteins is achieved through multiple pathways and multimeric complexes called translocases. One of the major ones dealing with more that 50% of all mitochondrial proteins is the TIM23 pathway with its critical components TIMM23 and TIMM17 forming the membrane core of the translocase complex. The TIM23 protein translocase plays a key role in establishing the mitochondrial proteome and also contributes to the dynamic changes within these proteome to respond to the needs of different cell types and to sustain the alternations in metabolism. The bulk knowledge about the activity and architecture of the TIM23 comes from a model organism yeast. The basic principles of protein import as well as the architecture of the translocase are likely conserved throughout evolution, but its mechanisms of action and regulation in human cells remain surprisingly unknown.

Recent works, including research from our group, have shed some light on the regulation of human TIM23 activity, opening a very exciting area of research. First, the most important component directly involved in the protein import is TIMM17, which exists in two variants in human cells: TIMM17A and TIMM17B. Their regulation is strikingly different: whereas TIMM17B is relatively stable and serves to sustain the function, TIMM17A is a short lived protein, highly regulated by degradation executed by the YME1 protease. **The goal of this research proposal is to address this significant gap in our knowledge and uncover the mechanisms governing the operation and regulation of TIM23 translocase in human cells.**

A special focus will be given to understanding the architecture and functional differences between the two forms of TIM23, containing either TIMM17A or TIMM17B subunits. We will characterize their ability to import mitochondrial proteins. The published work indicates that the two forms of the TIM23 core are likely to bind different components of the entire complex. The architecture of the TIM23 forms and their dynamics will be comprehensively characterized. Using advanced protein labeling technologies and proteomics we will uncover the global changes in mitochondrial proteins and the role of TIMM17 variants in shaping the plasticity of the mitochondrial proteome. Our group has proposed in the recently published work that the turnover of the short-lived TIM23 core translocase containing TIMM17A is highly regulated by a protein called OCIAD1. However, the mechanisms of this regulation remain unknown. Through systematic analyses undertaken within this project we will uncover the mechanisms regulating TIM23, involving biogenesis of the TIM23 complex at the levels of import, assembly, and degradation of the translocase complex components. **Our published discoveries, a plethora of unpublished data, already generated tools, and our expertise put us in a right position to deliver a comprehensive picture on how protein import through TIM23 is regulated and how it contributes to shaping mitochondrial form and function**. Without this knowledge it is impossible to understand human physiology, metabolism, and the deficiencies observed in many mitochondria-related diseases, spanning through cancer, metabolic diseases and age-related and neurodegeneration.