Every living organism needs energy to sustain its life and to reproduce. In humans, this energy is provided mostly by mitochondria, which are small powerplants that constitute an intrinsic and indispensable component of almost every cell. The dysregulation of these organelles is associated with numerous diseases, including cancer, neurodegeneration and aberrant inflammatory response. In order to fulfil its function, mitochondria import some proteins that are made elsewhere in the cell, for example polynucleotide phosphorylase (PNPase), a highly conserved enzyme catalysing RNA degradation. Human PNPase (hPNPase) resides mostly in the mitochondrial intermembrane space (IMS), a compartment situated between two membranes surrounding the mitochondrial matrix (Figure 1), but its function there is poorly understood. Some hPNPase is also located in the mitochondrial matrix, where the enzyme acts in a degradative mode that is supported by a complex it forms with another protein, Suv3, to destroy superfluous RNA (Figure 1).

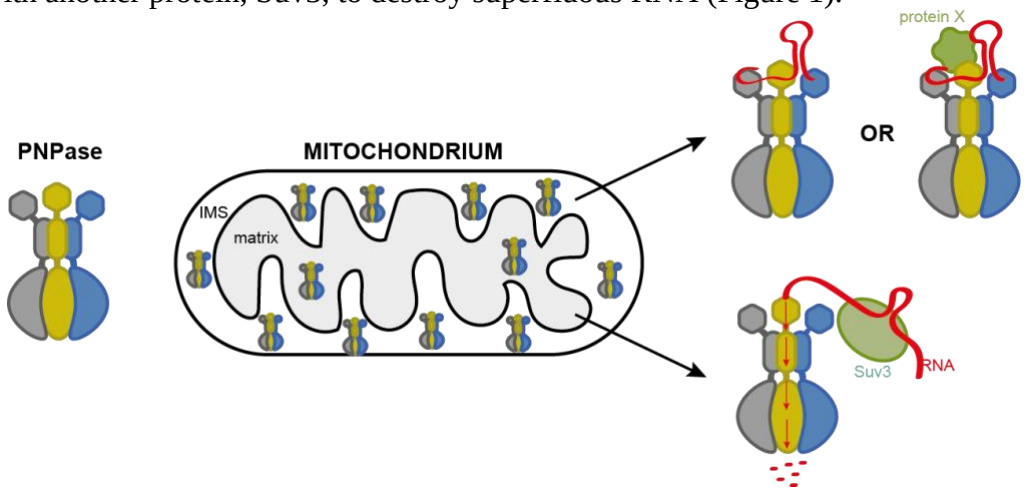

Figure 1. PNPase in the human mitochondrium. The left panel shows a schematic of PNPase structure. The middle panel shows the mitochondrial localisation of the enzyme. The enzyme has two proposed two modes of activity, namely protective (top panel, right side) and degradative (bottom panel, right side). Protein X is a hypothetical protein partner that may support the protective mode (details in text).

Bacterial PNPase was recently shown to switch activity from RNA degradation to a mode of RNA protection when complexed with another protein partner. As the human and bacterial enzymes are highly similar in sequence and structure, it is possible that hPNPase could have a dual mode of action as well, and this second activity could be used in the human mitochondrial IMS. The proposed protective mode would require either a special RNA substrate, that hPNPase would not be able to cleave, or another protein partner which would be responsible for switching off the hPNPase degrading activity (Figure 1).

My research plan aims at understanding the role and function of hPNPase in the intermembrane space of the mitochondria. My working hypothesis is that hPNPase displays two modes of action on its RNA substrates, and the protective one could be exploited functionally in the IMS, where the enzyme would be RNA-mediated regulator of mitochondria function. On the other hand, in the mitochondrial matrix hPNPase in complex with Suv3 would always act in a degradative mode (Figure 1). This way, by physical separation of hPNPase in two different compartments, both its activities could be supporting optimal performance of the mitochondria.

I would like to conduct biochemical and molecular biology experiments that will determine if hPNPase can function as an RNA regulatory factor as well as an RNA degrading enzyme. I also plan to determine the precise three-dimensional structure of hPNPase using cryo-electron microscopy, a rapidly evolving technique allowing scientists all over the world to gain insight into different complicated macromolecular assemblies. My planned research will help us understand the role of human PNPase in the mitochondrial intermembrane space, and with such knowledge we could be one step closer to recognise causes of some mitochondrial disorders.