# Chapter 2: Aims & Objectives

## 2.1 Rationale of hypothesis

The mutations of important proteins and genes in PD are the most important cause of the neuronal cell death, and the death of dopaminergic neurons is known to be a hallmark of PD, but the exact mechanism behind the pathogenesis of the disease is still elusive. Many efforts are being carried out to know the mechanism behind this neuronal death. Out of these, α-syn plays a significant role in the pathogenesis of the disease, and furthermore, the fact that α-syn may be transferred to bystander neurons through exosomes, and show harmful effects on the neighboring neurons, maybe a significant factor in the spread of the disease and the propagation of the disease. Neuronal degeneration and α-synuclein aggregation is observed in non-dopaminergic parts of the brain too, like, neocortex, brain stem and olfactory bulb [82]. Recent research indicates that exosomes play a significant role in facilitating communication between neurons and between neurons and glial cells in the brain [262]. In PD insulted neurons, α-synuclein is predominantly taken up in exosomes and are transferred to nearby neurons and leads to neuronal dysfunction [263]. These α-synuclein loaded exosomes are uptaken by the glial cells and induce inflammation [264]. This evidence suggests that exosomes play a major role in neuron-glial communication, however the intracellular pathway leading to the exosomal release in neuronal and glial cells in the PD stress conditions has not been well elucidated.

Given its vital role in the maintenance of the cell homeostasis and viability, mitochondrial dysfunction and abnormalities play a central role in PD pathogenesis. The dysfunction is mainly characterized by a decrease in the complex I activity of the OXPHOS assembly, generation of ROS, ATP depletion, and release of cytochrome c. The turnover of mitochondria through mitophagy (a kind of selective autophagy) is important neuronal survival including dopaminergic neurons [167]. On one hand, MVBs are known to fuse with the lysosome where their content is degraded and recycled back for the cell's use [192]. On the other hand, MVBs may also fuse with the plasma membrane, releasing the ILV content in the form of exosomes [193]. Lysosomal function may be crucial to this balance hence it is critically regulated through mitochondria and lysosomal crosstalk for the process of autophagy and cellular homeostasis. This suggests a crosstalk between the lysosome and mitochondria and exosome release in PD [265] which may play an essential role in pathogenesis of PD in bystander cells. This cross talk between mitochondria, lysosome and exosome in neuronal cells have not yet been investigated

systematically in PD stress conditions. In this study, we analyzed the mitochondrial-lysosome crosstalk in PD stress conditions and its role in exosome release from the cell in PD stress conditions.

Exosomes are also known to contain signalling molecules that are transferred to the nearby recipient cells which modulate the functional outcome of the recipient cells [266]. It is very well known that miRNAs regulate the gene expression in a cell at the post-transcriptional level, ultimately modulating the protein levels. Similarly, the miRNAs involved in the pathogenesis of the disease are not yet known, whether they are transferred to the neighboring cells via exosomes. The identification of the novel miRNAs that may be transferred to the neighboring cells via exosomes may help us understand better the pathogenesis of the disease and help in designing novel approaches to regulate the cell death for better management of the disease. We hypothesize that miRNAs that are implicated in the disease may be transferred through the exosomes to bystander cells and may cause mitochondrial dysfunction in the recipient cells. This may modulate the neurodegeneration and cell death that is observed in PD.

Hence, we propose to identify the miRNAs which may be transferred through exosomes to bystander cells, and find out the mitochondrial dysfunction occurring through these miRNAs, ultimately leading to cell death observed in PD. We aim to identify the novel miRNAs in exosomes that could regulate mitochondrial functions and dynamics in PD conditions, ultimately leading to neuronal death.

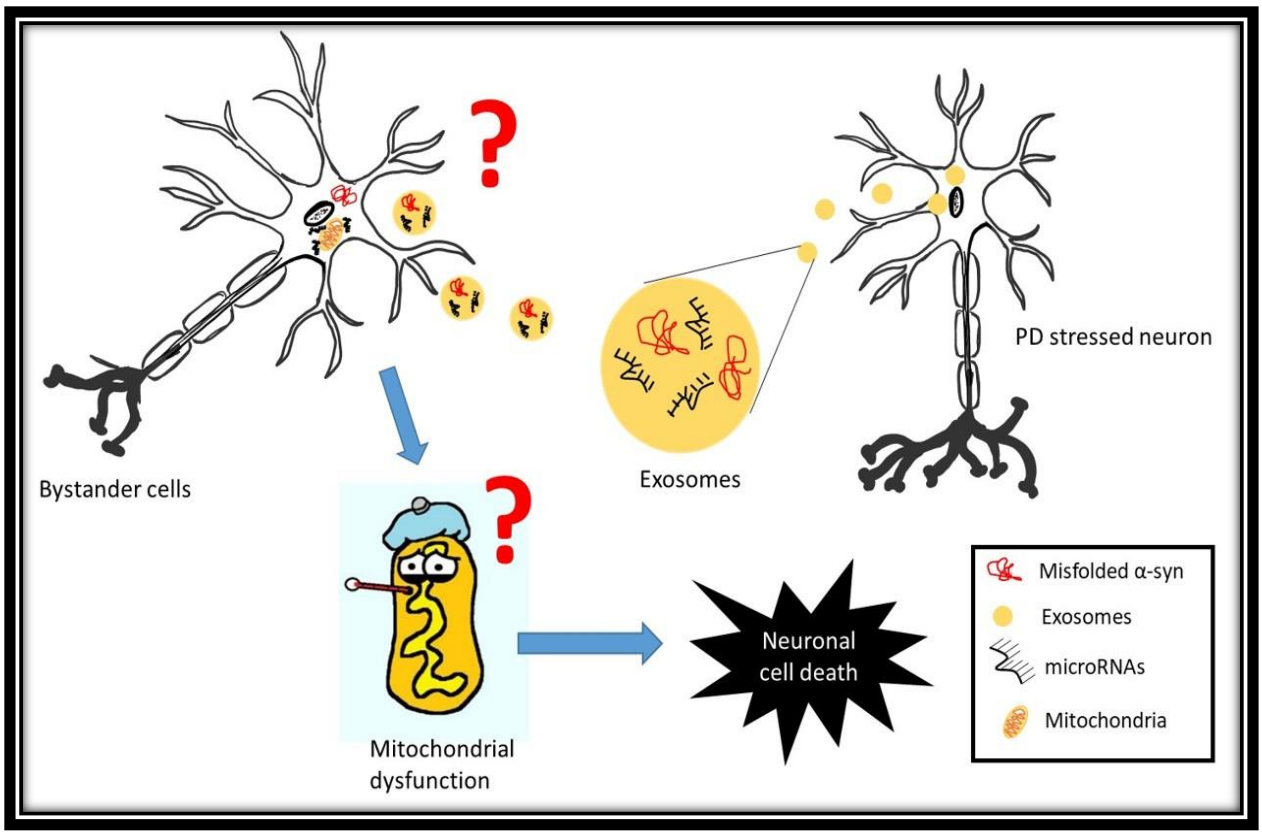


**Figure 2.1. Diagrammatic representation of the hypothesis of the study.**

## 2.2 Specific objectives

- Objective – 1: To study the effect of PD stress conditions on the release of exosomes, and their uptake in the bystander cells

- Objective – 2: To identify the exosomal miRNAs, their targets, and their alterations in PD stress conditions

- Objective – 3: To study the role of the identified exosomal miRNAs in mitochondrial functions and cell death in PD stressed cells as well as the bystander cells