Study of α-synuclein aggregate toxicity using a nanopipette delivery system

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In Parkinson's disease the protein α-synuclein aggregates to form amyloid fibrils in the cytoplasm of neurons. How the aggregation of α-synuclein in cytoplasm causes neuronal cell death is poorly understood. Most α-synuclein cellular toxicity studies have been limited to the addition of exogenous aggregates to the outside of the cells which does not reflect the toxicity caused by intracellular cytoplasmic aggregation of α-synuclein.

The nanopipettes are pipettes in nanometer scale, generally made from quartz capilliarise. The nanopipette is an advanced tool which allows the controlled voltage-driven delivery of individual exogenous molecules directly into the cytoplasm or nucleus of a single cell by integrating the nanopipette with the scanning ion-conductance microscopy. This tool is widely used to deliver DNA and fluorescent molecules, but not with α-synuclein aggregates. The α-synuclein aggregates passing through the nanometer-size nanopipette opening cause a temporary blockage of the ion current flow whose duration and magnitude depend on the size of the aggregate. We aim to use these signals to quantify and simultaneously analyze the nature of the aggregates while being delivered to the cytoplasm of a single cell.

By using the nanopipette, we aim to deliver defined numbers of structurally characterised α-synuclein aggregates (fibrils and prefibrillar oligomers) into the cytoplasm of single cells and study their effect on cell viability. We will then examine the aggregates' effect on cell viability. The successful completion of this project will not only provide information on type of aggregates that are toxic to cells, but also how many of each aggregate is required to kill a cell. This information allow us to better understand the pathology of Parkinson's disease in a single cell environment, and lead to better drug design in the future.