A “Cool” way to study Parkinson´s Disease

The Big Bang Theory left us amazed about how Leonard was able to bring back a snowflake embedded in resin from the North Pole to give to Penny. The reality is that this concept is not so far-fetched and a very similar process takes place in our laboratory on a regular basis.

Our work does not focus on snowflake research, but on small structures present within every cell of our body called autophagosomes. Autophagosomes remove unwanted rubbish from our cells in a similar way a vacuum cleaner cleans your home. These autophagosomes increase the longevity of cells and help remove unwanted bacteria or protein clutter. When this protein clutter accumulates in large numbers it destroys cells in our brains called neurons and leads to the onset of the most frequently reported neurodegenerative disorder, Parkinson´s disease. By determining how these autophagosomes form we can help define future therapies which may one day alleviate this condition.

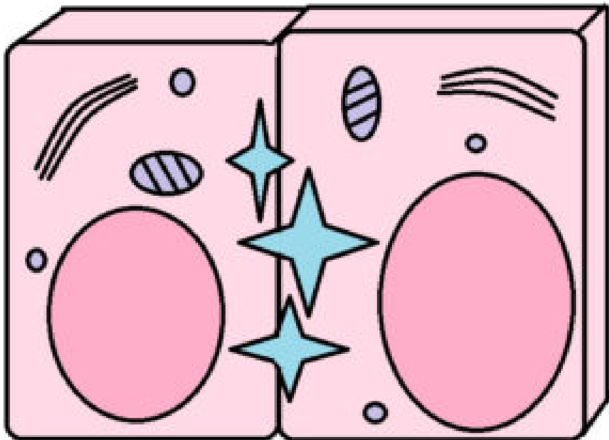

Fig. 1. If cells are frozen too slowly sharp cubic ice crystals form (blue stars) and damage the cell membrane.

Here we will describe how frozen cells can be preserved, embedded into resin and visualized using a microscope and how this is helping us gain insight into onset of the leading neurodegenerative disorder of the 21st Century.

Firstly, in a process called cryofixation, cells are grown on small sapphire discs and are loaded into a High-Pressure Freezer. This machine freezes the cells under high-pressure by rapidly plunging them into liquid nitrogen. Under normal atmospheric pressure, liquid nitrogen has a temperature of -196°C so it is the perfect candidate for super-cooling our cells. Due to the extremely rapid nature of the freeze, all of cellular the water (70 % of cells content) is unable to reorganise into a cubic

crystalline arrangement and therefore is unable to expand, like when you freeze water in your water bottle. If that water bottle was our cell, it would mean it would expand and burst into tiny pieces. Instead, all of the water forms another type of ice called vitreous ice taken from the latin word for `*glass*`.

In this frozen *glass* state, the delicate structure of the cell is preserved and a special machine which operates under -90°C substitutes all of the frozen water in the sample with solvents such acetone. When this step is complete, the cells can be embedded into resin. One method is to place the sample into resin which can be hardened in the oven at 60°C, alternatively resin can be hardened under freezing conditions. A trip to your local dentist can shed some `*light*` into this technique, particularly UV light, as some resins are able to be hardened by UV light, regardless of temperature.

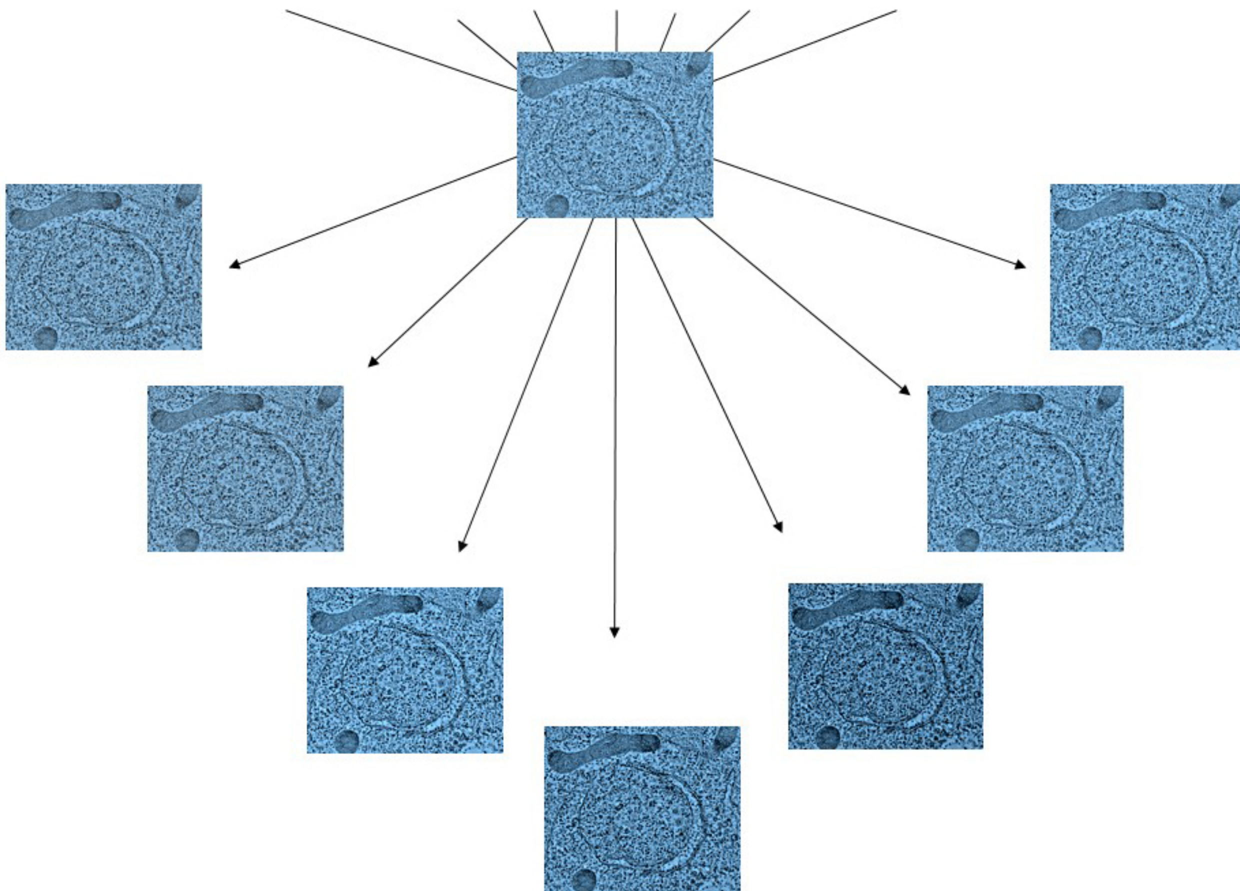

Fig. 2. Snapshots are taken at different angels to compile a final 3D volume of the cellular structure.

So now we have cryoimmobilized autophagosomes which are embedded in resin. How do we

visualize them if they are so small? Imagine you had to precisely outline a tiny circle on a page, what would you use? A fine felt-tip pen or a thick paint brush? This analogy compares light microscopy with electron microscopy. Although light microscopy allows you to resolve individual cells, it is the added power of electron microscopy which uses a beam of tiny particles called electrons that can help resolve small structures like autophagosomes. Electron microscopy has the added capabilities of electron tomography which allows you to take photos of the autophagosome at different tilting angles, a bit like taking time-lapse photography at different swinging stages of a pendulum. The final photos are then used to re-build a 3D model of the ultrastructural detail. It is this detailed research that is bringing us closer to discovering what promotes autophagosome formation and its role in the clearance of the protein clusters in neurons.

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Publication

Ultrastructural Characterization of Phagophores Using Electron Tomography on Cryoimmobilized and Freeze Substituted Samples.
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