3D Imaging Method to Visualize the Network of LRRK2-positive filaments in Cells and Investigate their Relation to Other Organelles

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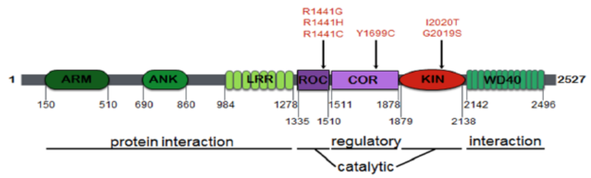
Abstract

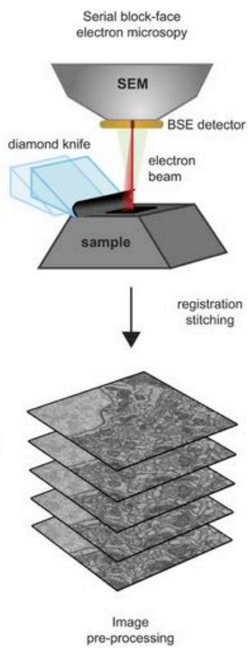
Leucine Rich Repeat Kinase 2 (LRRK2) is an enzyme found within cells. Mutations in this gene have been associated with Parkinson's disease (PD), a common neurodegenerative movement disorder characterized by tremor, rigidity and slowness of movement. More specifically, missense mutations in the enzymatic core of LRRK2 that affect the regular expression of this enzyme have been shown to cause familial PD. LRRK2 PD-related mutations enhance LRRK2 oligomerization, causing the formation of filamentous structures when these pathogenic proteins are expressed transiently in cell lines or primary neuronal cultures. The goal of this experiment was to take a sample of a wild-type LRRK2-expressing cell and track the location of LRRK2 in relation to other organelles such as the mitochondria of the cell and the nucleus. Through gathering data, then tracing and building a 3-Dimensional model of mitochondrial interaction with LRRK2, the research provided results that can later be compared to a cell expressing mutated LRRK2 proteins to see how the interaction differs. Though LRRK2 is not the only gene involved in familial Parkinson's cases (it accounts for 5 to 40% of the cases, depending on the specific populations), the similarity in effects can make the regular and mutated cell experiments important in gaining more information on the nature of neurodegeneration, including sporadic Parkinson's disease and the extent of change that is potentially caused to the mitochondria of the cell.

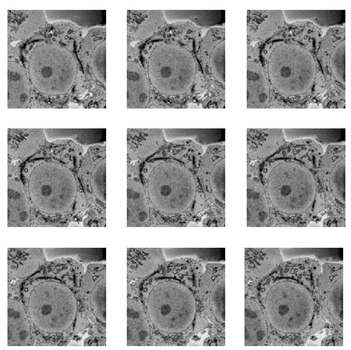
LRRK2 Mutation Background



The mutations involving LRRK2 have been found to mostly occur in the Roc-Cor-Kin (or enzymatic core) region (where the kinase domain is located, of which the G2019S is the most common mutation to occur). This mutation and many others enhance oligomerization of LRRK2 which leads to a greater formation of filaments within the cell. The filaments consists of bundles of microtubules decorated by LRRK2 proteins.

Microscope Imaging and Setting Up the Sample





The images on the right represent different sections of the sample collected using SBEM.

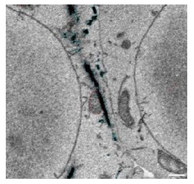
The first part of the process involved imaging the sample using a 3D technique called Serial Block-Face Scanning Electron Microscopy (SBEM). In this iterative process, a diamond knife (placed inside the microscope) cuts ultra-thin sections of the sample in the SEM and the resulting block-face (surface of the sample) is imaged, creating a series of sequential images that are then stitched together to generate a large scale 3D cellular reconstruction with nanometer resolution. Following thresholding and manual segmentation a final 3D volume was obtained, allowing to document the network of the LRRK2 filaments and the mitochondria throughout the cell. While the mitochondria can be identified based on morphology, it was important to apply a special EM probe that would mark the LRRK2 protein to make it visible by EM (thus the darker staining on the images above represents the LRRK2 filaments that have formed through oligomerization).

Tracing

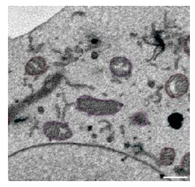
After the sample was stitched back together, it was important to trace and classify both the mitochondria and the LRRK2 to ensure that all the objects would be defined and visible when the 3D model was finally put together. Every section required tracing for all the objects present to keep track of all potential interactions and changes for the LRRK2 and various mitochondria.

Tracing the Mitochondria

Mitochondria that interacted, traced in red



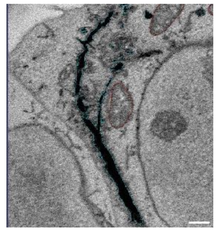
Mitochondria that did not interact, traced in purple



For the mitochondria, it was important to classify between those that were in close proximity to the LRRK2 filaments at any point (or on any contour) versus those that did not interact with the filaments at all. Due to the sectioning process, some mitochondria were split between several sections, and thus they appeared to split or reform in some contours during the tracing. The 3D model (which was forming parallel to the tracing) provided a clear view from different angles that helped distinguish which parts belonged to the same mitochondria and which did not.
(Scale bar is 420 nm)

Tracing the LRRK2

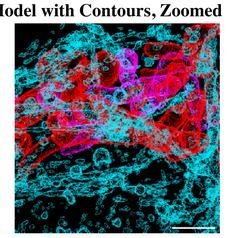
LRRK2 filament traced in blue



Even though it was important to trace the mitochondria carefully, a lot of the LRRK2 filaments were smaller and not as regularly shaped as the mitochondria, making it even more crucial to trace intricately to make sure that even the smallest potential overlaps (which are the interactions between the mitochondria and the filament-forming protein) would be accounted for, making it easier to classify each mitochondrion as one that did or did not interact with the LRRK2 filaments.
(Scale Bar is 420 nm)

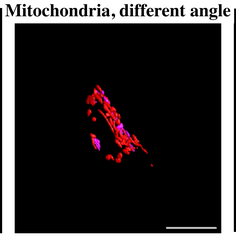
3D Model

Model with Contours, Zoomed in

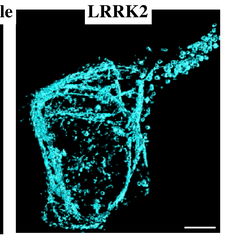


Mitochondria

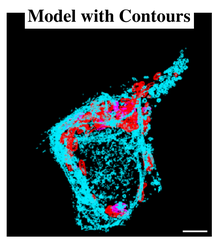
Mitochondria, different angle



LRRK2

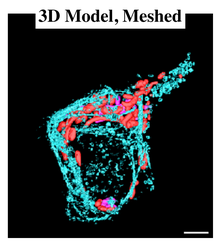


Model with Contours

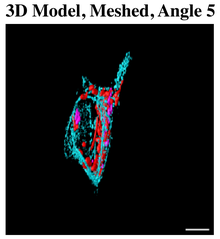


The 3D Model was the final step in the tracing process as it provided an account for all the different mitochondria based on classification as well as the LRRK2 protein, in addition to areas of close contact (which could be seen by rotating to different angles). It provided a view that was permanent as well, as it replaced having to go back through the different sections and contours to see the interactions and changes.
(Scale bar is 420 nm)

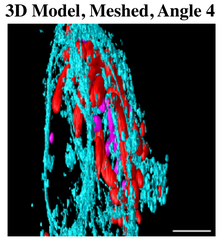
3D Model, Meshed



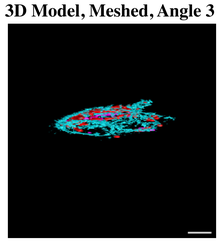
3D Model, Meshed, Angle 5



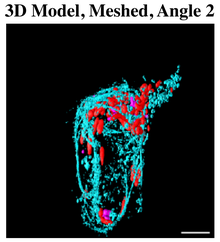
3D Model, Meshed, Angle 4



3D Model, Meshed, Angle 3

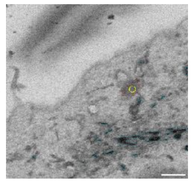


3D Model, Meshed, Angle 2

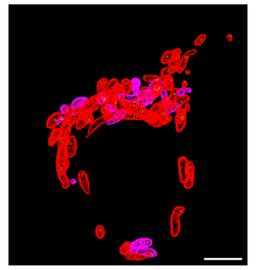


Data Analysis

Marker placed on mitochondria



To get the mitochondria classification data, a marker type object was used for one contour of each mitochondria to track the total number of mitochondria in the traced sample, after which looking at the 3D model helped determine the total number of markers as well as the breakdown of the results. (Scale Bar is 420 nm)



(Model View) All of the mitochondria in the sample, regardless of classification, are identified with the yellow marker

Tracking the Classification of the Mitochondria

	Contours	Number of Objects
Mitochondria (in contact with filaments)	1416	50
Mitochondria (no interaction)	416	18

Out of a total of 68 mitochondria, 18 (or roughly 26.5%) did not interact with the LRRK2 filaments, and 50 mitochondria (73.5%) were in close contact with the filaments. For future experiments it would be interesting to investigate PD-related LRRK2 mutant proteins and their propensity to form filaments and interaction with mitochondria within the cell.

Discussion and Conclusions

Mitochondrial dysfunction represents a critical event in the pathogenesis of PD. Previous studies suggested a direct role of LRRK2 in regulating mitochondrial dynamics and function. However, there are not reports on the high resolution visualization of LRRK2 proteins interacting with mitochondria. The initial assessment showed that by using a combination of EM probes and 3D imaging methods, I could clearly visualize the distribution of the wild type LRRK2 proteins in whole cell 3D reconstructions with nanometer resolution. Interestingly, I observed LRRK2 filaments in close contact with mitochondria. Specifically 73.5% of the mitochondria within the cell analyzed were in contact with LRRK2. While there were a few filaments in close proximity to mitochondria, most of the filaments were not near the nucleus of the cell where a considerable amount of the mitochondria were, which is why there were several mitochondria that were classified as those that did not interact. The experiment is still not conclusive in terms of complete results, as a second process of imaging and tracing must be conducted with a cell with mutant LRRK2 to see the changes in the behavior of the protein and filament production in this case, which will potentially give patterns and new insight into how the different characteristics of LRRK2 in the mutant version lead to the effects that cause Parkinson's disease. Since the propensity to form filaments is more pronounced in PD-related mutant forms of the LRRK2 protein, the next step is extending a similar analysis to LRRK2 mutants.

References

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2. [Diagram of the different LRRK2 Mutations and Protein Domains]. (n.d.). Retrieved July 24, 2017.

3. Serial Block-Face Electron Microscopy [Digital image]. (n.d.). Retrieved July 24, 2017.

Acknowledgments

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