## Section II: Methodology

### Role of Student vs. Mentor

While my mentor handled the mixing of 6-OHDA with OP50 due to it being a neurotoxin, I was able to complete the rest of the experimentation such as the assays and lab techniques. Additionally, my mentor assisted with the mathematical calculations when deciding the final concentrations for my experimental groups. The timeline for my project begins with brainstorming in the fall, followed by experimentation beginning in December for preliminary data.

### Equipment and Materials

The worm models Wildtype N2 and the NL5901 strain were used as controls. Initially, to further simulate Parkinson's symptoms by inducing oxidative stress, 6-OHDA was mixed with bacteria with a 20mM stock concentration. The NL901 strain was exposed to the neurotoxin via food source. Similarly, curcumin and gold-nanoparticles were mixed with bacteria for uptake by the worms in concentrations of 100mM. After this, behavioral assays were conducted on each of the experimental groups so the results can be analyzed and compared.

### 6-OHDA and Curcumin incubation

Since 6-OHDA needs to be dissolved to be mixed with OP50, ethanol was used to dilute it. Simultaneously, OP50 was spun down in a centrifuge until there was a pellet in the bottom of the tubes. Then, 10 ml of bacteria was mixed with 6-OHDA and LB broth into a solution. Lastly, a proportion of the bacteria solution was spread on NGM agar plates using pipetting techniques under the fume hood. Similarly, curcumin was mixed with OP50 in 100mM stock concentrations and then spread into NGM plates for the worms to uptake the treatment.

### Locomotion Assay

A locomotion assay is done to measure C. elegans' movement via counting their body bends per 20 seconds. In this case, the body bends were counted manually.

**Basal Slowing Response**

Basal Slowing Response is a worm's response to food. In the wildtype, it is expected that the worms are slower when approaching food than when they are travelling on plain NGM plates. Thus, body bends per 20 seconds were counted manually for when the worms are in the presence and absence of food.

**A-synuclein assay**

Since the NL5910 strain expresses YFP as a-synuclein in their walls, a fluorescent microscope is used to view the a-synuclein levels. Through the microscope, green light can be seen where the a-synuclein proteins are being expressed. To compare levels, Image J software was used to analyze the differences in florescence intensity.

**Statistical Tests**

ANOVA was performed on the experimental groups after each assay. It was done to determine if there was any significant difference among the groups. All tests were conducted on Excel.

***Student's t test***

If there was a significant difference in the ANOVA results, post-hoc Student t-tests were conducted to identify specific group differences.