Hello, my name is Elahson Swanson and I am a junior at Illinois State University studying biochemistry. Today, I am going to be talking about Small Molecule Iron Chelation for Parkinson's Disease Therapy. This is the research I was able to conduct during part of the spring 2020 semester as well as the summer of 2020 due to a research fellowship opportunity provided by Illinois State University's Office of Student Research. Before I begin, I would just like to thank the East Central Illinois ACS Undergraduate Research Conference for this opportunity and platform to present my research.

Parkinson's Disease is a progressive, neurodegenerative disease characterized by symptoms such as tremors, slowed movement, loss of automatic movement, and changes in speech. One of the primary causes of Parkinson's Disease is the death of dopaminergic neurons due to oxidative stress. Furthermore, another characteristic of Parkinson's Disease is the Parkinson's protein, alpha-synuclein, which is found in increased levels in the brains of those with Parkinson's disease and is depicted to the right.

Brains of those with Parkinson's Disease have been found to contain a relatively high level of iron. Iron is a redox active metal, which is able to produce reactive oxygen species that wreak havoc on the brain and produce the oxidative stress we observe in the brains of Parkinson's Disease patients. Additionally, Parkinson's Disease brains have been found to contain intracellular protein deposits known as Lewy Bodies, which are depicted in the image on the right. These intracellular protein deposits are comprised of alpha-synuclein and have been found to coordinate with iron at the His-50 residue. This coordination with iron on His-50 is a problem. There is an issue with the amount of iron present in the Parkinson's Disease brain, and in order to mitigate the effects of iron, our goal was to synthesize an iron chelator to sequester iron and prevent subsequent aggregation of alpha-synuclein and oxidative stress. What we are really looking at here is: does the chelator compete with the protein for iron?

In order to synthesize our desired complex, C1, we used a click chemistry reaction, and a design centered on the brain's high affinity for glucose. We used a sugar molecule as a tag to get into the brain, and a pyridine to coordinate with iron, like so. Our calculated molar mass of complex, C1, was 308.29 grams per mole. Our mass spectrometry results showed a mass to charge value of 309.11993; this indicates that we were indeed able to synthesize our desired complex. As expected, the mass to charge value was one gram per mole higher than the molar mass due to the protonation occurring during mass spectrometry because mass spectrometry detects cations. So, once we knew we had synthesized our desired complex, we were then able to characterize it via several methods to determine its ability to coordinate with iron in solution and its favorability to be incorporated intracellularly.

In order to characterize our synthesized complex, C1, we used several methods such as Log-D, UV-Vis, ThT fluorescence, dynamic light scattering, and transmission electron microscopy.

One of the first tests we did was the Log-D test. The Log-D test detects a property called lipophilicity, which is a drug property that predicts a compound's solubility, absorption, and membrane penetration. Log-D stands for the logarithm of the phosphate buffer saline 1-octanol partition coefficient. We used PBS with a pH of seven-point four due to physiological pH being seven point four. The value we found for C1 was negative one point two five eight. This value

indicates that C1 has greater solubility in aqueous solutions compared to hydrophobic solutions. So, in our experiment we found that C1 had a greater affinity for the phosphate buffer saline than the 1-octanol. This value lies in a range that indicates that C1 should be permeable to a biological membrane. And, this is due to Lipinski's Rule of Five. Lipinski's Rule of Five is a set of general guidelines to aid development of bioavailable drugs. These guidelines suggest that a Log-D value between negative point 5 and positive five is favorable. While our calculated value lies slightly outside of these limits, it is not a large concern because this value is likely due to the glucose tag on our molecule, and we know that glucose can access the brain. Furthermore, Lipinski's Rule of Five suggests that a molecular weight limit of 500 Daltons is favorable as well, which we have also achieved with this complex.

The next test we did was UV-Vis in order to compare the absorbance spectrum of complex one with the absorbance spectrum of complex one combined in solution with iron two and iron three. We are interested in this because a change in the absorbance spectrum may indicate that our complex is coordinating with iron in solution. Here our data shows a slight change in absorbance spectrum of complex one compared to complex one combined with iron two or three in solution. However, in both the case of complex one combined with iron two and complex one combined with iron three, we see the same lambda max as complex one. Based on these results, it is unclear if iron is coordinating with complex one in solution, so we needed further evidence to determine what exactly is going on in solution or draw any conclusions from this data.

The next test we did was a ThT fluorescence biological assay, and this is one of our most important pieces of evidence. What this data looks at, is the amount of fluorescence, which correlates with relative aggregation of the alpha-synuclein protein. To conduct this experiment 1:1 mixtures of protein, metal salt, and chelator were prepared in phosphate buffer saline with a pH of seven point four. Thioflavin T was added in order to produce the fluorescence. We incubated samples of alpha-synuclein, alpha-synuclein plus complex one, alpha-synuclein plus iron two or iron three, and alpha-synuclein in solutions with both iron two or three and complex one for 72 hours. We measured the fluorescence every 24 hours. Our percent relative aggregation we are looking at is relative to the amount of 100% of alpha-synuclein aggregation. What we see here is the more fluorescence we have, the more aggregation we have.

Looking at the graph on the left, we have the graph of thioflavin T fluorescence with iron three. The 100% aggregation is representative of the aggregation of alpha-synuclein alone and is depicted in the blue. The percent relative aggregation of alpha-synuclein plus complex one is represented in orange, and the percent relative aggregation of alpha-synuclein plus iron three is depicted in the grey. Lastly, they yellow represents our synthesized complex combined in solution with alpha-synuclein and iron three. Here we see that our percent relative aggregation of alpha-synuclein and iron three is a bit lower than expected. This is peculiar due to what we know about alpha-synuclein and iron in the brains of Parkinson's disease patients; when we have alpha-synuclein and iron together we usually have a lot of aggregation. However, we still see from this data that when we added our complex into solution, depicted by the yellow line, we still see less than 100% relative aggregation until approximately sixty hours of incubation, which is still a good indicator that our complex is decreasing alpha-synuclein aggregation. But further experimentation of these solutions may provide a better indication of what exactly is going on in solution

Looking at the graph on the right, here we have the thioflavin T fluorescence of iron two. Here we see a similar trend but, in this case, we see that the relative percent aggregation of alpha-synuclein and iron two together is much higher. When we look at the solution of alpha-synuclein, iron two and complex one, this shows a large decrease in aggregation. We see a decrease down to approximately thirty to forty percent relative aggregation, and much less aggregation than that of alpha-synuclein and iron two alone. This is a great sign and indicates that our complex is coordinating with iron in solution and preventing aggregation of alpha synuclein.

So next, we took those same samples from the fluorescence assay and analyzed them using DLS and TEM. DLS, or dynamic light scattering is a spectroscopy method used to determine the size distribution of particles. In this method, a single frequency laser is directed towards the sample, and this incident laser light is scattered. The scattered light is detected at a certain angle over time and the signal collected is used to determine particle size. This is based on the Strokes-Einstein equation which gives the relation between the speed of particles and their size. Looking at our graph on the left of size vs intensity, the black peak is representative of the particle size of alpha-synuclein, and the red peak represents the particle size of alpha-synuclein and iron three, and this makes more sense as we see shift to larger particle sizes, and thus more aggregation when we add iron in solution with alpha-synuclein. Then, we can see that when we add in complex one into solution with alpha-synuclein and iron, we see a shift to a smaller particle size, depicted in the blue. This indicates that our complex is coordinating with iron, preventing alpha-synuclein aggregation and is thereby able to produce a smaller particle size. Likewise, we took those same samples and looked at them using transmission electron microscopy, or, TEM, and this is a microscopy technique in which a beam of electrons is transmitted through a sample in order to form an image. The top image we can see here is our image of alpha-synuclein and we see these spindly little aggregates which is typical of what we would expect. And then once we add in iron three to our alpha-synuclein we see a ton of aggregation, which once again is exactly what we would expect knowing what we know the brains of Parkinson's patients and the aggregation we see in the Lewy Bodies when we have alpha-synuclein coordinating with iron. Then looking at the last imagine we see alpha-synuclein and iron three with complex one. This is a wonderful piece of data as it shows a significant decrease in aggregation when complex one is incorporated. It is a great indicator that our complex is coordinating with iron in solution and decreasing alpha-synuclein aggregation.

Likewise, we also have the DLS and TEM data for the iron two containing solutions. Here we also see a shift to a smaller particle size when we include complex one into solution with alpha-synuclein and iron two. This is depicted in the graph on the left. We also see a decrease of aggregation in our TEM images when complex one was incorporated as well, as we observe the diminished intensity of the last image of alpha-synuclein, iron two, and complex one, which indicates that there is less aggregation. Once again, this in an indication that our complex is coordinating with iron in solution and decreasing alpha-synuclein aggregation.

Our evidence from our thioflavin T biological assay, DLS, and TEM, suggests that complex one coordinates with iron in solution and is thereby able to decrease aggregation of alpha-synuclein. Our evidence from our Log-D experiment suggests that complex one is a biologically suitable structure, as well as what we know about its molecular weight lying within favorable parameters. Thus, we can conclude that complex one is a promising candidate for a therapeutic agent for Parkinson's disease. And next, we will be looking at derivatives of complex one and evaluating their ability to coordinate with iron and decrease alpha-synuclein aggregation

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