Hello everyone, my name is Cade Meiss. I am a senior student at Illinois State University. I will be doing a presentation on Ruthenium based therapeutics for Alzheimer's research. I have been working on this during the past two semesters of school. I would like to take this moment to thank the East Central Illinois ACS conference for giving me this opportunity to speak.

Alzheimer's disease is a neurodegenerative disease currently found in some of our aging population most commonly people over the age of 65. Around 6 million people have cognitive impairment due to AD, which is characterized by a slow loss of memory over a long period of time until an individual can no longer create new memories, nor remember old ones. In the end stages, a person with Alzheimer's is completely reliant on others for all activities of daily living. The disease begins with the incorrect cleavage of the Amyloid precursor peptide into two smaller peptides, one of which is the AB peptide. The peptide then slowly transforms into a partially folded state, and then into an aggregate called an oligomer. These oligomers are used as a base to form longer aggregates called protofibrils and fibrils. The dangerous and cytotoxic part of the disease has been found to be the oligomer. Focus has been on keeping this oligomer out of solution, thereby decreasing its cytotoxicity. The Aβ peptide has been shown to aggregate alone or to metal centers and these aggregates become plaques that stop the transmission of information along the neuronal pathway. There are some symptom relief drugs, but until this point, there is no therapeutic that has been approved by the FDA to slow or stop the progression of the disease. And as you can see here, this is the Amyloid Beta peptide. We will talk more about the specific use of therapeutics on the Aβ peptide later.

Metal based drugs were not very researched or used until the success of cisplatin, which you can see in the top left corner. After the success of cis-platin as an anticancer drug, the research into metal-based drugs has increased significantly. The goal of using metal-based compounds as an Alzheimer's therapeutic is to prevent the aggregation of Aβ, thereby decreasing the formation of Aβ plaques. Originally more platinum compounds were tried and found to have success inhibiting the AB peptide in mice. Since then, more metal centers have been researched. Ruthenium based compounds like the one shown here have shown promise in inhibiting the AB peptide aggregation. Our research lab has been looking into ruthenium-based compounds.

When looking at the ligands to coordinate to the metal complex, there are a lot of options. Our research lab had previously used pyridine coordinated compounds, so we wanted to try something similar. The two compounds I made had been made previously for anticancer research. Quinoline compounds like clioquinol had also been previously used and shown promise. Between the fact that the compounds had been previously synthesized and similar ligands had shown promise, we were interested in the effects of our proposed compounds on the AB peptide. Compound C2 was made using the starting compound and 8-amino quinoline in a 1:1 ratio dissolved in dichloromethane and stirred for 4 hours. Compound C1 was synthesized using the starting compound and 8-hydroxyquinoline in a 1:1 ratio. The compounds were combined with sodium methoxide in methanol under a nitrogen atmosphere and stirred for four hours. Upon synthesis of the compounds we ran Electrospray ionization mass spectrometry to get these mass to charge ratios. The mass to charge ratio is about 35m/z below the molar mass of the compound because the spectrometer was in positive mode meaning the negatively charged Cl ion

was not in the m/z ratio. This, along with our hydrogen NMR allowed us to ensure these compounds were synthesized as expected.

The goal of the ruthenium compounds is to target one of the three histidines on the peptide. We believe it does this through the Chlorine leaving and then the compound coordinates to one of the nitrogens on the histidine residue. In coordinating the residue, the complex would then inhibit the aggregation of the peptide. The phenol group on the top section of the ruthenium forces the compound to have a single specific location to coordinate. The aromatic rings of the quinoline are hydrophobic and will interact with the hydrophobic region of the peptide.

UV-Vis spectroscopy was important in determining how well the compounds remain dissolved in a phosphate buffer saline, which is at a similar pH as our bodies. The compounds were dissolved in DMSO and then diluted in PBS. The UV-Vis was ran at one scan every 10 minutes for 6 hours. The compound C1 does not change over a six-hour time frame indicating it remains in solution. C2, however, can be seen to quickly go through some sort of change after just 10 minutes, with some of it precipitating out of solution. This is a concern, as a therapeutic that cannot remain in solution to reach the target would not be very useful. One change that is important to see is the ligand exchange where the chloride would be lost from the compound.

In order to determine how the two compounds modulate the aggregation of the Amyloid beta peptide, a ThT biological assay was completed. The AB peptide, ThT, and compounds were all dissolved in stock solutions and diluted to a concentration of 10um with filtered PBS in the well. Each sample was created in quadruplicate, and then incubated with shaking for 24 hours before being measured for fluorescence. This graph represents the relative fluorescence of the compounds with AB in solution vs. The amyloid beta peptide by itself. Here we can see the reduction in fluorescence due to the coordination of the peptide with the metal complex. C1 has a fluorescence 7.7% the magnitude of the Aβ, and C2 has a fluorescence of 0.45% that of the Aβ at the excitation wavelength of 404nm and emission of 477nm.Other ruthenium compounds we have been analyzing have been reducing the fluorescence to 20-40% that of the AB. According to our test, the reduction in fluorescence is phenomenal. As a side note, we found that once coordinated to the AB peptide, compound 2 remains in solution much better.

After running the ThT biological assay, the Dynamic light scattering of the wells is run. On top of this a Transmission electron microscopy plate is created with the same plate. This makes the data extremely consistent, as all of the data came from the same set of solutions. DLS is a measure of the fluctuation of light as it passes particles as they move randomly in solution due to Brownian motion. It is used to determine the size distribution of a solution. Smaller particles move faster than larger ones, and that distribution can then be found through the scatter of a laser and is graphed on an autocorrelation curve. Three different scans are overlayed in the graph. Each sample was run in three times and the average can be seen. This shows the changes when the metal complexes coordinated to the AB peptide. Without modulation the AB peptide has a size of about 320nm where the two compounds can be seen to both decrease the size and intensity of AB peptide. The presence of a second peak for C1 indicates the peptide is being broken down into smaller pieces. This is a Good sign, because the goal of the therapeutic is to decrease the size of the aggregate.

In conclusion, both compounds have promise as AB aggregation inhibitors. The biological assay fluorescence data shows strong inhibition of the AB peptide, especially for C2. Yes, this data does have some fairly large error bars, but the data came from a single test meaning more tests would reduce those error bars. The DLS data backs it up showing size reduction. The UV-Vis concerns for solubility can be assuaged by the fact that the compound is shown to remain in solution once coordinated to the peptide. These compounds, while showing promise, require more tests. Looking forward, the TEM plate from the Biological Assay has been sent out but we are waiting for results. A cytotoxicity screen towards glial cells will allow us to ensure the metal complexes are not cytotoxic.