Phosphorus NMR, Peptide Synthesis, and Metal Binding

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My group had a few different projects that we were focusing on. The first project we tackled was to finish constructing a lab to help bridge the gap between the sophomore class organic chemistry, also known as chem 262, and a junior class biochemistry, also known as chem 323. At Calvin organic chemistry is required to take the biochemistry class. The problem is that these classes build on each other but use very different techniques to gather data and reach conclusions. Our solution is to create this lab that uses phosphorus NRM spectroscopy, a rigorously used organic chemistry technique, to gather data on fundamental biochemistry molecules. This combination of organic chemistry and biochemistry should bridge the gap nicely and make an easier transition between the classes. We provided this project with the reference NMR data and worked on the write up for the lab.

The other project that took up a majority of the summer was working with experiments focusing on Alzheimer's disease. We are testing a hypothesis about how metal binding to peptides effects them and aggregates them into one of the hallmarks of Alzheimer's of plaques in the brain. First we synthesized our six amino acid long peptides. Our peptides are unique to our purposes in the fact that they are the smallest peptide we can create with up to tertiary structure. They formed a beta-hairpin loop. Another unique feature of our peptides is that they have a non-natural amino acid on them called dopamine. This amino acid is the same as the very common amino acid tyrosine but dopamine is oxidized. The structure of our protein simulate protein in the brain and the dopamine simulates oxidative damage in the brain that is accumulated over time as a person gets older. Also on our peptides we have a variable site that we changed to create six distinct peptides. In this site we changed the amino acid to be naturally occurring and differ in its size and chemistry. This was to see if oxidative damage is the cause of metal binding.

After we synthesized these six peptides we used the HPLC (high pressure liquid chromatography) to purify our proteins. After purification we used NRM and MALDI mass spectroscopy to identify and categorize our peptides to make sure that we made the correct thing. Once this was completed we moved on to the experiments. First we made up solutions combining our different peptides, metals (iron, aluminum, copper, and zinc), and a carbon matrix to use with the MALDI. This showed us whether or not our peptides were complexing with these metals. We found that they were but we needed better tests to see how they were complexing and what the effects on the peptides were.

Lastly we made solutions of our peptides for the NMR. At first we took scans without metal of all of our peptides. Then we titrated metal into the NMR samples to get data for different concentrations of metal and peptide. We concluded that the metal ions were binding to the dopamine. This validated our hypothesis. We also found that with increasing amounts of metal there are shifts in the NMR spectrum that are interesting and the rest of the summer will be spent exploring those shifts.

This research opportunity benefited me greatly in deciding what I want to do after college. I now know for sure that I do like research and would be happy pursuing a career in the science research field. But another thing is that working closely with my mentor all summer has brought me is talking about graduate school. This was never really on the table for me as a thought for after college. There is a strong chance now for me to pursue that interest right out of college instead of finding a job right away. This opportunity was also great to gather research experience as an undergraduate.