Spring 2019 Research Report:

Progress, Discussions, and Future Plans

This semester consisted of several projects going on; with Brandon leaving, I had a lot to learn about everything that he does. We started out the semester by continuing the construction of the new fluorescence microscope. The microscope, however, is still not finished; hopefully some of the Doc Kieft researchers will play around with it and possibly finish it before I get back next semester. Otherwise, it will most likely be finished during the Fall 2019 semester.

During this time, I began to run some more trials with insulin. I also began working more with Brandon with the Brewster Angle Microscope (BAM). Brandon and I have been making adjustments with the BAM and I have been learning how the entire set up works so that I can make improvements to it later on and potentially build a new BAM out of metal to increase the accuracy and reproducibility of the data collected.

During this semester, I also had the opportunity to present mine and Kate's data at the 2019 National ACS Conference in Orlando, Florida. This was an awesome experience being able to go to several interesting talks such as one that studied the interaction dynamics of phospholipid monolayer systems using NMR. It was a great opportunity to begin practicing how to present my data and explain it to people who may or may not have any experience with the topic.

Once we got back from the conference, Brandon and I began work for a paper to be submitted to a special edition of the Journal of Inorganic Biochemistry in honor of Debbie Crans. Some of the trials I collected for the paper focused on insulin on a water subphase, which I believe now to be the dimer and not the monomer due to some stability papers; I also did trials

on a $ZnCl_2$ subphase and on a $ZnCl_2$ and EDTA subphase. All of these trials were performed at specific pHs, all of which were focused around insulin's isoelectric point, 5.4. In the beginning of trials, I thought that the isoelectric point of insulin was 5.8, so I made solutions at ph 3, 5.8, and 9. I ran into problems with this, however, due to solubility issues with $ZnCl_2$ and EDTA. At high pHs, $ZnCl_2$ tends to form ZnO or ZnOH and precipitates out of solution. On the other hand, at low pH, EDTA forms little crystals that also precipitate out of solution. After rethinking some things, and realizing that the isoelectric point of insulin is 5.4, I decided to plan on the following. I never got to do it though since we stopped writing the paper. So, the following are some future plans that I, or maybe Kyle, will do:

- Insulin on Water Subphase:
    - pH: 3.4, 5.4, 7.4
- Insulin with $ZnCl_2$ dissolved on Water:
    - pH: 3.4, 5.4, 7.4
    - Note: This is different than normal since adding $ZnCl_2$ into insulin solution and not into the subphase
- Insulin with $ZnCl_2$ dissolved on EDTA:
    - pH: 3.4, 5.4, 7.4
    - Note: I decided to try this method to get an insight into insulin's dissociation and see if we could visualize the dissociation of insulin from a hexamer to a monomer
- All of this is to be imaged using the BAM

- We would also play around the the temperature, since all of these are currently being done at 25℃. It would be interesting to see what, if anything, would change if it were done at physiological temperature, 37℃.

I would still like to work towards publishing this work in a journal some time. I think it would be nice to have all of the data mentioned above, but also the following data to get an insight into not only insulin's aggregation and dissociation, but also how it interacts with other lipids at various pH:

- Insulin with DPPC:
    - pH: 3.4, 5.4, 7.4
- Insulin with DOPC:
    - pH: 3.4, 5.4, 7.4
- Insulin with Cholesterol:
    - pH: 3.4, 5.4, 7.4
- Insulin with DPPS:
    - pH: 3.4, 5.4, 7.4
- Insulin with Sphingomyelin:
    - pH: 3.4, 5.4, 7.4
- Ideally all of this would also be imaged with the BAM

With all of this data, I think would could get a very holistic view of insulins aggregation and interactions with the cell membrane as well as its pH dependence for each of those aspects.