It was a great opportunity to be a part of 2018 NSFs ChemMatCARS Faculty and Student Team Research Award (FaSTRAC). The award was in the form of a month-long internship and a 4 day beamtime, primarily to interact with the beamline staff and run experiments at the Sector 15 ID-C related to my ongoing PhD Thesis work. I am currently in the 2nd year of the Chemical Engineering PhD Program at the City University of New York, City College (CCNY). I am co-advised by Dr. Raymond Tu (Associate Professor, Chemical Engineering Department) and Dr. Charles Maldarelli (Professor, Levich Institute) and we are working on understanding the phase-behavior and dynamics of the monoclonal antibodies (mAbs) and excipients at the air-water interface.

We came to know about this program via an email from our Associate Dean for Research, Dr. Rosemarie D. Wesson. As the notice was forwarded well ahead of time, I had a chance to grab this opportunity and set up the goals for this month-long internship. Dr. Binhua Lin and Dr. Wei Bu, played an important role during this process to help understand and guide us about the application process and how it can benefit us in the long run. The application for this Award was a combination of Curriculum vitae, a brief proposal for the research project, statement about career advancement and a LOR from the advisor. At CCNY, we perform experiments on different interfacial tools such as Pendant bubble tensiometer, Langmuir-Blodgett trough, Brewster Angle Microscope and some characterization tools namely Circular Dichroism and Fourier Transform Infrared Spectroscopy (FTIR). With the help of these tools we are able to understand the adsorption of mAbs and excipients on a molecular scale. This answer questions like is the adsorption process reversible or irreversible, what are the time scales for the adsorption of these molecules, help determine the secondary structure of the mAbs and a generalized Equation of State for mAbs and excipients system. Although these interfacial tools can give us qualitative answers about our system, but it is more promising to get some quantitative data on how thick the adsorption layer is at the air-water interface, how is the competition between the mAbs and excipients taking place and how much surface coverage is occupied by these systems. This is where X-ray can be a powerful tool to help understand the features at an Angstrom scale. Our goal was to link the established interfacial techniques with the X-ray reflectivity studies to gain a whole picture of our mAb excipient system.

I had an opportunity to visit Sector 15 ID-C under a Rapid General User Proposal for a 24-hr. beamline in October 2017. With the results obtained during this beamtime, it helped us to understand the structure and behavior of the protein and excipient at the air-water interface. Since, it was a 1-day beamtime, I had less time to interact with the beamline staff, even though Dr. Bu was kind enough to help us understand to analyze our data via email after our visit. We were able to connect the results with the ongoing interfacial techniques, as mentioned above. In order to complete a whole set of experiments we had in mind, applying for this internship was definitely a good opportunity.

The one-month stay at ChemMatCARS was well organized and I was supervised by Dr. Wei Bu, Dr. Mrinal Kanti Bera and Dr. Binhua Lin. My first week was more about understanding this high energy tool via reading some articles suggested by the beamline scientists. The suggestion of reading the book “Liquid Surfaces and Interfaces: Synchroton X-ray Methods” by Peter Pershan and Mark Schlossman was definitely worth it. It helped me to understand the basics about the X-Ray Reflectivity, Grazing Incidence X-ray Diffraction (GID) and Grazing Incidence Small Angle X-ray Scattering (GISAXS). I remember correctly, there were some instances that I used to get stuck in the heavy math while reading those articles. But nonetheless,

Mrinal and Wei, were able to catch this situation and used to stop by my desk while walking in the hallway and help me understand it in an easier way. This in-turn helped me to understand the minute details in the derivation of Fresnel Reflectivity , Patterson Function, Parratt Method and so on. This later was eventually helpful during data analysis. At the end of my first week, I had a chance to discuss in detail with the beamline scientists about the list of experiments I had in mind to perform for the 4 -day beamline period. Their suggestions to modify some of the experiments so that I can use those 4 days of beamtime efficiently. Helped me to gather data in a more systematic manner. I feel this was a great opportunity that I got to talk with the beamline scientists in-person way ahead of my beamtime schedule.

During the start of the second week, it was the time to start my 1st beamtime to understand the behavior of DPPC molecules at the air-water and air-histidine salt interface. Prior to the start of the experiment, the beam was off and so Wei had to adjust all the parameters using the ApexII software in order to ensure that the beam is in position, the detector is working and the slits are in their correct position before the start of the run. This was indeed a great learning opportunity for me. As a general user, exposure to this setup is usually not the case. As a part of the internship, Wei helped me to understand how the beam intensity is adjusted to make sure everything is in position and is in working condition. Luckily or unluckily there was some problem and we were not able to see the reflected beam on the detector. The trouble shooting procedure by Wei to fix this problem was indeed a good experience. It was then time for my first beamtime. He guided me through the procedure of what needs to be ensured before switching the beam on, such as searching the hutch, making sure the feedback, vibrational table and the helium flow was on. Furthermore, Wei helped me to understand the macros for each technique (Reflectivity , GID and GIXOS). He walked me through each step in the macro to understand what the function of each python command line is. This helped me during my upcoming beamtime to modify some steps in the macro, for instance to run a particular scan, if the beam goes off in between the scans. I was able to input the command lines to ensure that the scan is started from where it was left when the beam turned off. As it is said, X-ray time and energy should not be wasted. Keeping this goal and making sure that all the planned experiments were completed was a major task. The rest of the week was spent in analyzing the data using the Data Analyzer software based on Parratt's Method. As per Wei's statement, getting data is crossing the first hurdle, the next step is to analyze the data". This was true, analyzing the data was a major task. However, Wei and Mrinal helped me to understand it in a more systematic manner.

The rest two weeks, I had one of my advisor, Prof. Raymond Tu, to help me with the future experiments at the beamline. We discussed and refined our plan for the systems we want to characterize. Briefly, our first aim was to understand the co-adsorption of mAbs and excipients at the air-water interface. This was to identify what is the excipient concentration limit in the mAb sufficient enough to prevent the adsorption of protein using two different excipients, Polysorbate 80 and Pluronic F68. Our second aim was to understand the dynamic phase behavior of mAbs from very low concentration (0.0007 mg/ml) to high concentration (1 mg/ml). This helped us to understand whether the mAbs are undergoing some structural rearrangements at the air-water interface and whether their adsorption leads to multilayer formation. It was an exciting opportunity as a student to work with the advisor during these runs which helped us draft a path to couple our interfacial tools and X-ray techniques.

In addition, I was able to interact with few other users using liquid scattering technique at the Sector 15 ID – B. Interacting with these users, introduced me to other areas of science and realized how strong this tool is to get insights into systems. During my stay, I also got a chance to help one of the other interns who was joining from July at the University of Chicago. While transferring the knowledge which I received from the beamline scientists, I was able to help the student to understand the macro, trouble shoot problems if the beam goes off, how to interpret the data and so on. This was a good learning experience again, and I was able to go back and forth to talk with the scientist if some things were not clear. Furthermore, it helped me to be engaged so that I was able to pull off the night shift run ☺

As a student researcher, this internship has definitely opened a new area of science for me. There is a lot to explore more with this high energy technique. While taking a walk through the APS ring, I was thrilled to see the ongoing research at different sectors, including rheology, soft matter, thin films, interfacial science, biomolecules crystallography, and many more. I am currently working with Wei, Mrinal and Binhua to analyze the data on our systems. We look forward in publishing this data coupled with our interfacial techniques in near future and will be returning as a general user at Sector 15- ID, to perform and decode some other exciting insights of the mAb-excipient system.

Last but not the list, I take this opportunity to thank the staff at NSF's ChemMatCARS Sector 15. Many thanks to Dr. Binhua Lin, Dr. Wei Bu and Dr. Mrinal Bera for all the help during this internship. The interaction with the scientist was both fun and intellectual. I would also like to thank, Kimberly Simms for helping us with the lodging, ordering and shipping of materials. Furthermore, many thanks to Prof. Raymond Tu for his help during the beamline experiments and the interactions that we had to draft this work into a publication. Also, not to forget the fun we had by biking around the Argonne area. I look forward to work with Sector 15 and continue this relationship in the future.

Thank you ☺

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