NDnano
Center for Nano Science and Technology

ND*nano* Summer Undergraduate Research
2018 Project Summary

1. Student name & home university:
Erin O'Brien, University of Notre Dame

2. ND faculty name & department:
Dr. Matthew Webber, Department of Chemical and Biomolecular Engineering

3. Summer project title:
Improving Drug Efficacy through Supramolecular Affinity

4. Briefly describe new skills you acquired during your summer research:
I learned the process behind organic syntheses, as well as how to obtain and analyze data using electrospray-ionization mass spectrometry to identify the compounds in a mixture. I learned how to operate various machines that are often used in laboratory settings, such as a rotary evaporator, centrifuge, lyophilizer, and an analytical balance. Finally, I learned proper laboratory safety practices and improved upon my technical writing.

5. Briefly share a practical application/end use of your research:
The ability to prevent the aggregation of insulin for at least four days at ambient conditions will prove useful to the industry, as the transportation and storage of insulin has proved a difficult job to undertake. Synthesizing macrocycles with various appended reactive functional groups will allow for the Webber Lab Group to do further research into facilitating drug delivery and/or designing new drug capture technologies.

6. 50- to 75-word abstract of your project:
The use of reversible, non-covalent supramolecular interactions affords routes to materials with tunable function for a variety of applications.[1] Certain host macrocycles afford high affinity binding for complementary small molecule guest motifs.[2] One such interaction with the *N*-terminal phenylalanine on insulin was shown to promote enhanced stability, solubility, and efficacy of the protein when a macrocycle was covalently fused to a hydrophilic polymer.[3] To determine the utility of this approach as a platform excipient for the stabilization of many different protein drugs, we have worked to devise assays for real-time monitoring of protein aggregation. Leveraging the change in optical density of a protein upon aggregation, we replicated stressed storage conditions and determined the stability of insulin and related proteins under these conditions. Additionally, we developed a novel synthetic route to modify macrocycles,

[1] Webber, M. & Langer, R. (2017). Drug delivery by supramolecular design. *Chemical Society Reviews, 46*(21), 6600-6620.
[2] Webber, M. & Langer, R. (2017). Drug delivery by supramolecular design. *Chemical Society Reviews, 46*(21), 6600-6620.
[3] Webber, M., *et al*. (2016). Supramolecular PEGylation of Biopharmaceuticals. *PNAS 113*(50), 14189–14194.

for exploration of its use in conjugation chemistry. These modified macrocycles will expand the use of supramolecular chemistry to facilitate new drug delivery or drug capture technologies.

7. References for papers, posters, or presentations of your research:

1. Webber, M. & Langer, R. (2017). Drug delivery by supramolecular design. *Chemical Society Reviews*, *46*(21), 6600-6620.
2. Webber, M., *et al*. (2016). Supramolecular PEGylation of Biopharmaceuticals. *PNAS 113*(50), 14189–14194.
3. Webber, M., Appel, E., Meijer, E., & Langer, R. (2015). Supramolecular biomaterials. *Nature Materials*, *15*(1), 13-26.
4. Schlein, M. (2017). Insulin Formulation Characterization—the Thioflavin T Assays. *The AAPS Journal*, *19*(2), 397-408.
5. Harris, J.M. & Chess, R. (2003). Effect of pegylation on pharmaceuticals. *Nature Reviews Drug Discovery*, *2*(3), 214-221.

One-page project summary that describes problem, project goal and your activities / results:

Supramolecular chemistry uses the non-covalent interactions of molecules to prepare tunable materials with a variety of applications. In this project, we are using supramolecular chemistry to improve the efficacy of insulin and synthesize a new molecule to be used in further research efforts in the Webber Lab. Insulin has been a difficult compound to transport and use as it tends to aggregate within hours at ambient conditions, making it no longer useful to those who need it. In certain parts of the world, it is incredibly difficult to deliver, as proper storage conditions are scarce. Therefore, improving the stability of insulin by preventing aggregation would be extremely helpful to those who transport and use insulin. Along with improving the stability of the drug insulin, the Webber Lab also researches the use and optimization of other drugs using supramolecular chemistry. Using organic macrocycles, the lab experiments with host-guest chemistry to engineer drug excipients and drug delivery platforms. To expand the potential applications of these macrocycles toward improving modern drug technology, we sought to develop novel methods to append various reactive functional groups to monofunctionalized macrocycles for use in conjugation to further materials in the future.

Today, protein drugs play a significant role in treating disease. One such protein drug, insulin, is used in the treatment of diabetes by helping the body regulate blood sugar levels. Approximately nine percent of people living in the United States have diabetes, so the need for a reliable source of insulin at a reasonable price is necessary. However, because insulin needs to be refrigerated constantly in order to prevent the drug from losing proper peptide function, transportation and storage is both expensive and energy-inefficient. Therefore, the ability to decrease the cost of transportation of the drug by stabilizing it at ambient conditions would be beneficial. It has been suggested to promote the stability of these protein drugs by covalently modifying the protein with a synthetic polymer, often times (poly)ethylene glycol (PEG). By increasing the molecular weight of the protein and protecting the protein from proteolytic enzymes, the PEG chain prevents aggregation of the protein drugs at ambient conditions. Some organic macrocycles are known to bind to amino acid side chains displayed on the surface of insulin and other peptide drugs; by covalently modifying such macrocycles with a PEG chain, peptides in solution can be stabilized with these macrocycles to ease complications in protein storage and transport. An assay was constructed that mimicked physiological conditions, at pH 7.4 and 37°C, with constant agitation. The results of the assay show that the insulin alone aggregated at about ten hours, making the insulin no longer useful. However, the insulin that was mixed with PEG-modified macrocycles did not aggregate for at least one hundred hours, while peptide activity was maintained. The ten-fold increase in stability of insulin is very helpful in storing and transporting the protein drug.

Because the lab is researching drug delivery and drug capture technologies, synthesis of the macrocycles with various appended reactive functional groups will be helpful to the lab in conjugating these macrocycles to other molecules, drugs, or drug delivery platforms. By beginning with a macrocycle monofunctionalized with a short alkane chain and terminated with a single reactive functional group, a variety of subsequent reactive functional groups could be used to substitute the terminal group of the alkane chain, potentially expanding the chemistries amenable to these macrocycles in future projects. Each reaction was monitored using electrospray ionization mass spectrometry ((+)ESI-MS) and nuclear magnetic resonance (^{1}H-NMR) to detect conversion through multi-step reactions from starting products to intermediates and ultimately to final products. Through this basic research approach to developing new macrocycles, synthetic conditions were continuously optimized, accounting for reaction stoichiometry, solvent effects, solubility limitations, pH effects, and variable temperatures. Through this work, the Webber Lab can further investigate the use of these macrocycles in the use of drug capture and drug delivery technologies.