NDnano
Notre Dame Nanoscience and Technology

NDnano Summer Undergraduate Research
2022 Project Summary

1. Student name & home university:

Belen Flores, Saint Mary's College Notre Dame, Indiana

2. ND faculty name & department:

Dr. Matthew J. Webber, Department of Chemical and Biomolecular Engineering

3. Summer project title:

Glucose Responsive Glucagon for Hypoglycemia Rescue

4. Briefly describe new skills you acquired during your summer research:

During my summer research I learned how to use the following instruments: peptide synthesizer, liquid chromatography-mass spectrometer (LC-MS), rotary evaporator, zeta potential analyzer, and a nephelometer. Additionally, I developed my lab safety, pipetting, centrifuging, and record keeping skills.

5. Briefly share a practical application/end use of your research:

Designing and synthesizing glucose responsive glucagon for hypoglycemic individuals could potentially provide a safer and more reliable form of treatment than current commercial solutions. The glucose responsiveness of materials has been measured in the lab, but its efficacy must be tested on hypoglycemic mice. Upon success, studies could potentially move to human trials in hopes of creating a more effective form of treatment for hypoglycemia.

6. 50- to 75-word abstract of your project:

This project focuses on synthesizing a glucose responsive material as a preventative strategy for hypoglycemia rescue by incorporating glucose-sensing functionality into glucagon delivery. Zeta potential measurements and turbidity tests suggest resulting approaches have glucose-responsive function. However, further improvements must be made to increase sensitivity and ensure function. Future work thus will seek to improve glucose responsiveness and determine its efficacy in diabetic mice under severe hypoglycemia.

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

(1) Gelenter, M. D.; Dregni, A. J.; Duan, P.; Hong, M. Structurally Based Design of Glucagon Mutants That Inhibit Fibril Formation. *Biochemistry* 2021, *60* (25), 2033–2043.
(2) Liu, M.; Zhao, P.; Uddin, M. H.; Li, W.; Lin, F.; Chandrashekar, C.; Nishiuchi, Y.; Kajihara, Y.; Forbes, B. E.; Wootten, D.; Wade, J. D.; Hossain, M. A. Chemical Synthesis and Characterization of a Nonfibrillating Glycoglucagon. *Bioconjugate Chemistry* 2021, *32* (10), 2148–2153.

Glucagon is a naturally occurring hormone produced in the liver. In a healthy body, glucagon is released from the pancreas when blood glucose levels (BGLs) are low. This causes glycogen stored in the liver to break down into glucose and be released to elevate BGLs back to healthy ranges. However, people who experience severe hypoglycemia do not have enough endogenous glucagon to elevate BGLs back up to normal. As a result, exogenous glucagon is used during severe hypoglycemic episodes to rescue individuals from a coma or death.

Although exogenous glucagon may be used to treat severe hypoglycemia, two major issues remain. One issue is that natural glucagon has tendencies to fibrillate and become inactive when present in aqueous solutions at physiological pH. The second issue is that exogenous glucagon has to be self-administered or administered by another individual such as a caregiver. This is of major concern since the individual may be unconscious or sleeping when in need of treatment. The first issue has been tackled in previous studies by modifying glucagon to be more stable and soluble at physiological pH conditions through the attachment of a glycogen moiety. This reported modification has proved to have no influence on the activity of glucagon. Switching gears, this project specifically focused on addressing automated hypoglycemia rescue by creating a glucose responsive glucagon that could be administered nightly before bed.

To accomplish this, naturally occurring glucagon was modified and formulated to include glucose-sensing functionality. Additionally, some modifications were made to increase the stability of the molecule and reduce fibrillation. Ideally, the formulation would be insoluble and inert when BGLs are within normal ranges in the body. However, the formulation would be soluble, active, and functional once BGLs drop to low ranges in the body. This is because we want glucagon to be active only when needed.

My activities in the lab included working with graduate student Sihan Yu on synthesizing glucagon and peptides of interest. I attended weekly lab group meetings and subgroup meetings where our project would receive feedback and direction from our advisor Dr. Matthew J. Webber and other labmates. Additionally, I culminated the summer research I had participated in during the summer by presenting it at my final lab group meeting for the program. From this presentation, I was able to receive valuable feedback that I then incorporated into future presentations of the material.

In regards to the material of interest, experiments in the lab included synthesizing glucagon with various modifications using solid-phase methods and a peptide synthesizer. The protein was then cleaved from the resin and collected for further purification on a Biotage Isolera system. Purity of glucagon was verified by electrospray ionization mass spectrometry. The purified fractions of glucagon were placed in a rotary evaporator to separate it from the organic solvent present and then lyophilized . The solubility functionality of the obtained glucagon formulation was then evaluated by zeta potential measurements and a turbidity test.

Zeta potential measurements were taken at varying glucose concentrations (0, 200 mg/dL). The results indicate the formulation is soluble in the absence of glucose while insoluble in the presence of glucose. However, zeta potential measurements only provide a rough approximation of the sample's solubility. For this reason, the solubility was more directly tested using a turbidity test. Results of this analysis indicate some function, but the formulation is not sensitive enough to work under physiologically relevant glucose concentrations. Thus, future work includes improvement of glucose responsiveness and evaluation in a hypoglycemic mice model.

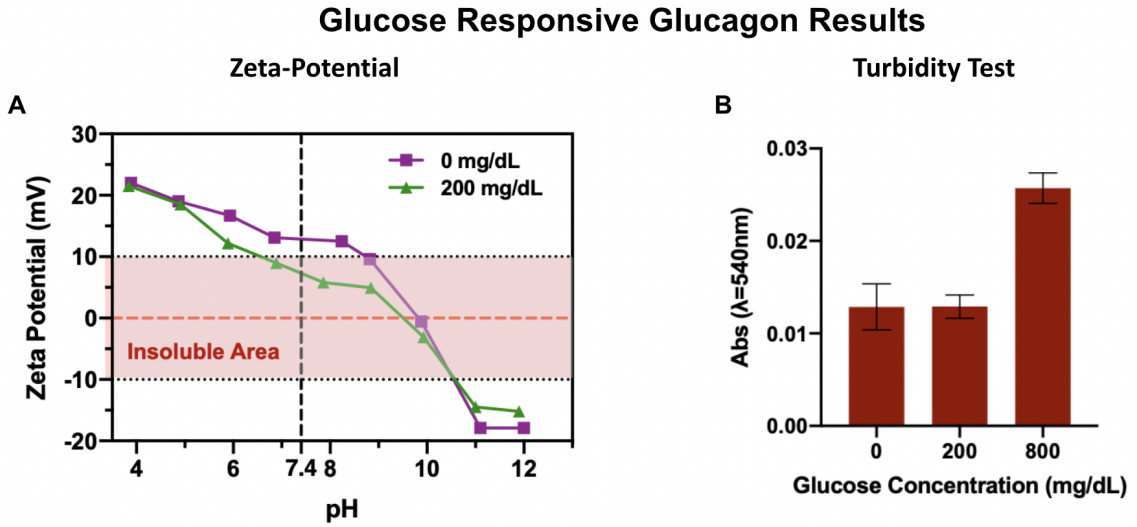

Figure 1. (A) Zeta potential measurements for glucose responsive glucagon under glucose concentrations of 0 and 200 mg/dL. (B) Turbidity test for glucose responsive glucagon in glucose concentrations of 0, 200, and 800 mg/dL.