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Notre Dame Nanoscience and Technology

NDnano Summer Undergraduate Research
2023 Project Summary

1. Student name & home university:
Elizabeth Power, University of Notre Dame
2. ND faculty name & department:
Dr. Matthew Webber, CBE
3. Summer project title:
Nanoscale formulation of corrective hormones for blood glucose control
4. Briefly describe new skills you acquired during your summer research:
Over the summer I was trained in automatic Solid Phase Peptide Synthesis (SPPS) on the Liberty Blue Peptide Synthesizer as well as hand coupling of peptides. I was also trained on Column Purification using Reverse Phase Chromatography to purify product, and Mass Spectrometry (MS) and NMR for confirmation of product.I was taught peptide characterization using Alizarin Red S (ARS) assays for functionality of boronate, Circular dichroism (CD) for evaluation of secondary structure, Isothermal titration calorimetry (ITC) for binding studies, and *In vitro* Ca^{2+} assays for glucose responsivity. Additionaly, I was trained in animal studies by the Freimann Life Science Center for handling and injecting mice.
5. Briefly share a practical application/end use of your research:
The development of a glucose responsive GLP-1 delivery would allow for safer and more stable regulation of blood glucose levels than currently available medications.
6. 50- to 75-word abstract of your project:
The GLP-1 delivery was designed such that function would be suppressed when blood glucose levels were low.Successful synthesis of the peptide was verified through mass spectroscopy and assays were performed to determine glucose-responsive structural and functional properties. Success in producing a glucose-responsive GLP-1 delivery method would introduce a safer therapeutic for individuals with T2D, allowing for improved glycemic control.
7. References for papers, posters, or presentations of your research:
(1) Springsteen, G.; Wang, B. Alizarin Red S. as a General Optical Reporter for Studying the Binding of Boronic Acids with Carbohydrates. *Chem. Commun.* **2001**, No. 17, 1608–1609. https://doi.org/10.1039/B104895N.
(2) Gallwitz, B. Anorexigenic Effects of GLP-1 and Its Analogues. *Handbook of experimental pharmacology* **2012**, *209*, 185–207. https://doi.org/10.1007/978-3-642-24716-3_8.
(3) *Figure 1. Standard CD spectra redrawn from Corrêa et al.[5] Each of the...* ResearchGate. https://www.researchgate.net/figure/Standard-CD-spectra-redrawn-from-Correa-et-al5-Each-of-the-three-basic-secondary_fig14_266950207 (accessed 2023-07-19).
(4) BPS Bioscience, Inc. *GLP-1R and Diabetes*. https://bpsbioscience.com/glp-1r-diabetes (accessed 2023-07-17).
(5) Manandhar, B.; Ahn, J.-M. Glucagon-like Peptide-1 (GLP-1) Analogs: Recent Advances, New Possibilities, and Therapeutic Implications. *J Med Chem* **2015**, *58* (3), 1020–1037. https://doi.org/10.1021/jm500810s.
(6) Zhao, Z.; Tang, Y.; Hu, Y.; Zhu, H.; Chen, X.; Zhao, B. Hypoglycemia Following the Use of Glucagon-like Peptide-1 Receptor Agonists: A Real-World Analysis of Post-Marketing Surveillance Data. *Annals of Translational Medicine* **2021**, *9* (18), 1482–1482. https://doi.org/10.21037/atm-21-4162.

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

Recent medical advances have highlighted the efficacy of Glucagon-like peptide 1 (GLP-1) analogs as a treatment for type 2 diabetes (T2D). This naturally occuring hormone acts to lower glucose levels by promoting insulin secretion and suppressing the release of glucagon. GLP-1treatements have also been shown to effectively lower BMI by sustaining satiety and slowing gastric emptying.While these two effects of GLP-1 make it a promising potential treatment for T2D, the pairing of GLP-1 treatments with other blood-glucose-lowering therapeutics that individuals with diabetes rely on, such as insulin, has been shown to significantly increase an individual's risk for hypoglycemia.

To address this issue, we have incorporated mechanisms to enable glucose-dependent delivery of GLP-1, such that function can be retained when needed but shut off when not needed, thereby lowering the risk of hypoglycemia. GLP-1 is composed of two alpha helices linked by a short linker region. We identified modification sites near the C-terminus, N-terminus, or within the linker region, that were determined not critical for receptor activation or binding based on alanine scanning studies. To achieve this synthesis I was trained on the Liberty Blue automatic peptide synthesizer as well as hand couplings of peptide. I was then trained on mass spectroscopy and NMR to verify the successful synthesis of the desired product, and column purification using reverse phase chromatography in order to purify the product.

In order to observe the functionality of the modifications we made I was trained in several characterization tests. An Alizarin Red S (ARS) assay was performed. This assay allowed us to observe the ability of our peptide to bind sugars. Circular dichroism testing was done to verify the alpha-helical structure of the peptide, and further tests were done at varying glucose concentrations in order to observe how the molecule changes conformationally when glucose is present.

Over the course of my summer I also received training in Isothermal Titration Calorimetry experiments, which could be utilized in the future with this project to conduct binding studies between our synthesized peptide and glucose. Additionally, I was trainined in Ca^{2+} assays which can be used to observe the extent of the glucose responsivity of a molecule. While assisting on other projects, I gained experience in animal studies, particularly in demonstrating hypoglycemic rescue in diabetic mice, which would be transeferrable to this project.

Success in producing a glucose-responsive GLP-1 delivery approach would introduce a safer therapeutic for individuals with T2D dependent on insulin therapy, allowing for improved glycemic control and satiety without fear of hypoglycemic incidents. The current data is promising and encourages us to continue with further research and development in this area.