Protecting Therapeutic Peptides From Proteolysis with Branched Structures using Click Chemistry

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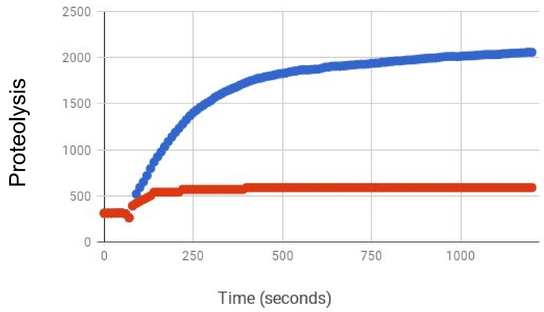
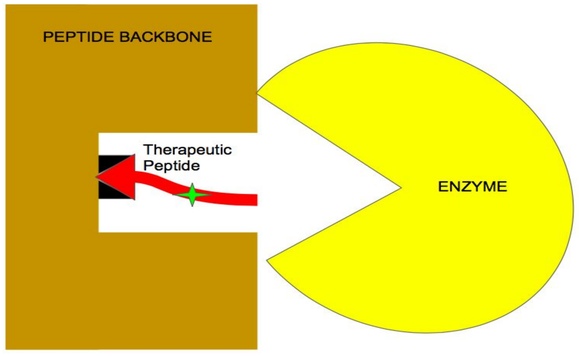
Introduction

Therapeutic peptides are natural compounds with fast clearance and low toxicity that could help with diseases such as Type-2 Diabetes; however, they are vulnerable to human body's natural enzymes and are quickly digested once entering the body, a process called proteolysis. Therefore they are currently only capable of short term treatment.

The goal of this project is to create a branched structure so the therapeutic peptides will be hidden and thus protected from the protease.

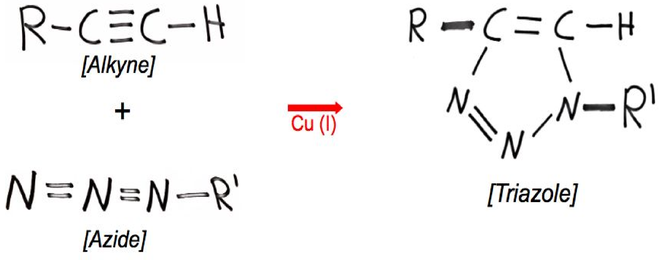


Blue Line represents the proteolysis rate of an unprotected peptide.
Red line represents the theoretical proteolysis rate of a protected peptide.

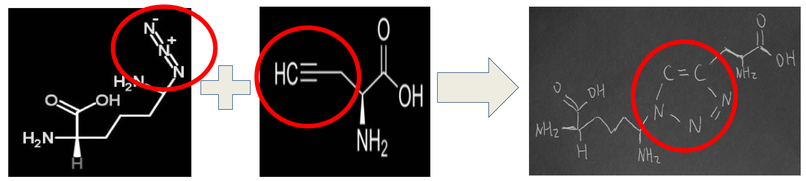


There are two steps in this model: building the peptide backbone and attaching a mock therapeutic peptide onto this backbone.

"Click Chemistry" was first brought up by Professor Sharpless and is used to attach two different chemicals with specific and corresponding structure. In this case, we are trying to click together an azide and an alkyne to form a triazole.



Amino Acids



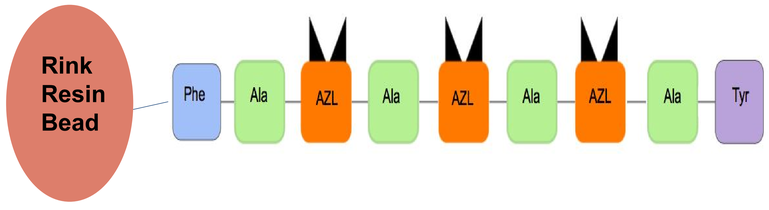
Azido Lysine

Propargylglycine

Alkyne is part of the amino acid Propargyl Glycine and Azide is found in Azido Lysine.

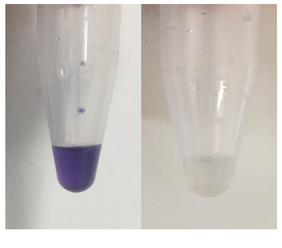
We decided to put Propargylglycine on the mock therapeutic peptide and Azido Lysine on the peptide backbone. This will enable click reaction between the mock therapeutic peptide and the peptide backbone.

Solid Phase Peptide Synthesis



Sequence of The Peptide Backbone
Phe-Ala-AZL-Ala-AZL-Ala-AZL-Ala-Tyr

Solid phase peptide synthesis uses Resin beads as the foundation of the peptide. We added each amino acid one by one and peptide bonds are formed between each amino acid and eventually complete this sequence.



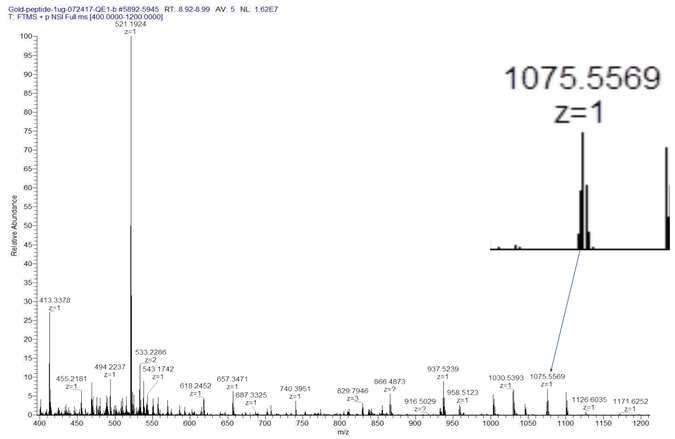
Kaiser Test

After coupling each amino acid, we performed a Kaiser Test.

A purple result indicates the presence of free Amine, which means we did not successfully attach the amino acid.

A clear and transparent Kaiser Test indicates our success at coupling the amino acid so that we can proceed to the next amino acid knowing the current state of the peptide.

Mass Spectrometry Analysis



Mass Spectrometry of Peptide

The peptide has a mass of 1073.5544 Da. The peak at 1075.5569 Da has a 2.32 ppm error. This is within the reasonable error, so it is very likely the peptide we desire.

However, the peptide concentration is significantly low.

So the desired peptide was successfully synthesized, but it was not synthesized well enough for further experiments.

Future Plans

Due to the arduous nature of solid phase peptide synthesis by hand, we have decided to order a peptide backbone from a company that can synthesize the peptide with an automated synthesizer.

After successfully synthesizing the peptide backbone, we will perform click reaction between the peptide backbone and the mock therapeutic peptide and measure the proteolysis rate of the product.

References

Blum, Angela P., Jacquelin K. Kammeyer, Jian Yin, Dustin T. Crystal, Anthony M. Rush, Michael K. Gilson, and Nathan C. Gianneschi. "Peptides Displayed as High Density Brush Polymers Resist Proteolysis and Retain Bioactivity." *Journal of the American Chemical Society* 136.43 (2014): 15422-5437. Web.

Rostovtsev, Vsevolod V., Luke G. Green, Valery V. Fokin, and K. Barry Sharpless. "ChemInform Abstract: A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes." *ChemInform* 33.43 (2010): n. pag. Web.

Acknowledgments

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