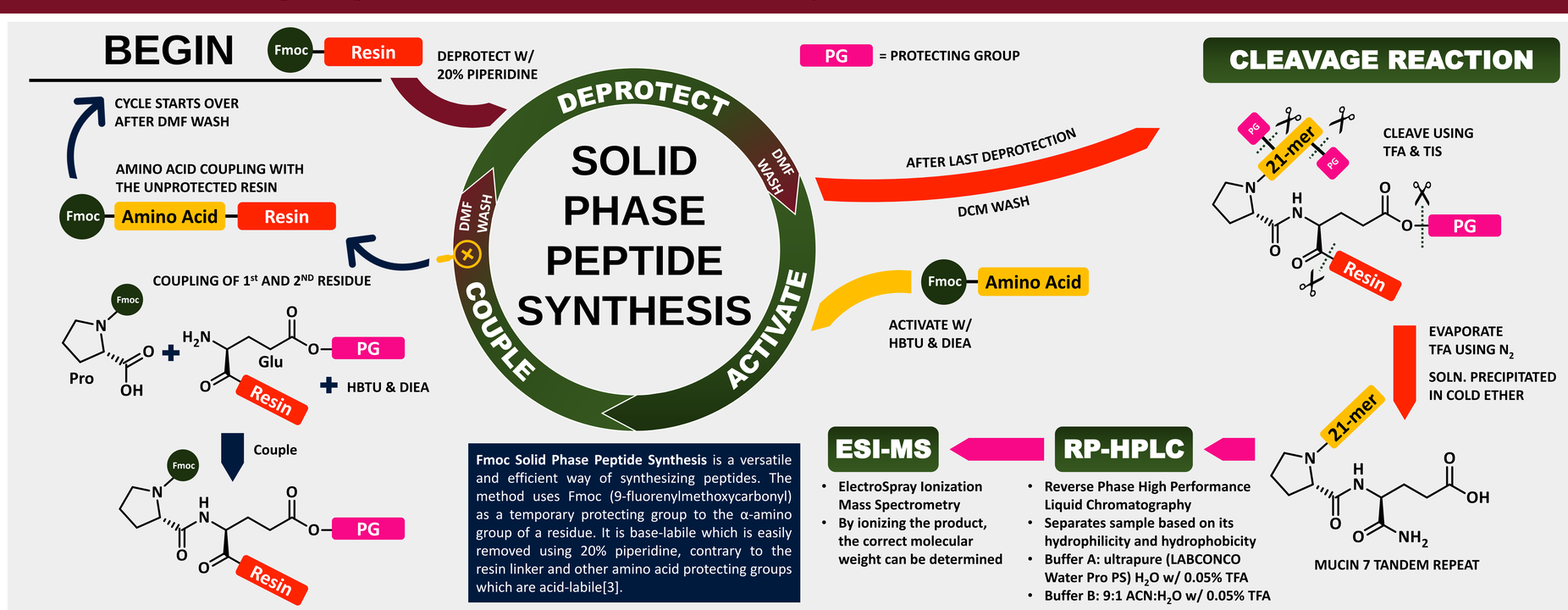
The synthesis of Mucin 7 tandem repeat

John Sanchez[1], Kristopher Waynant[2,3], Dharmeshkumar Patel[2,3], Darren Thompson[2,3]

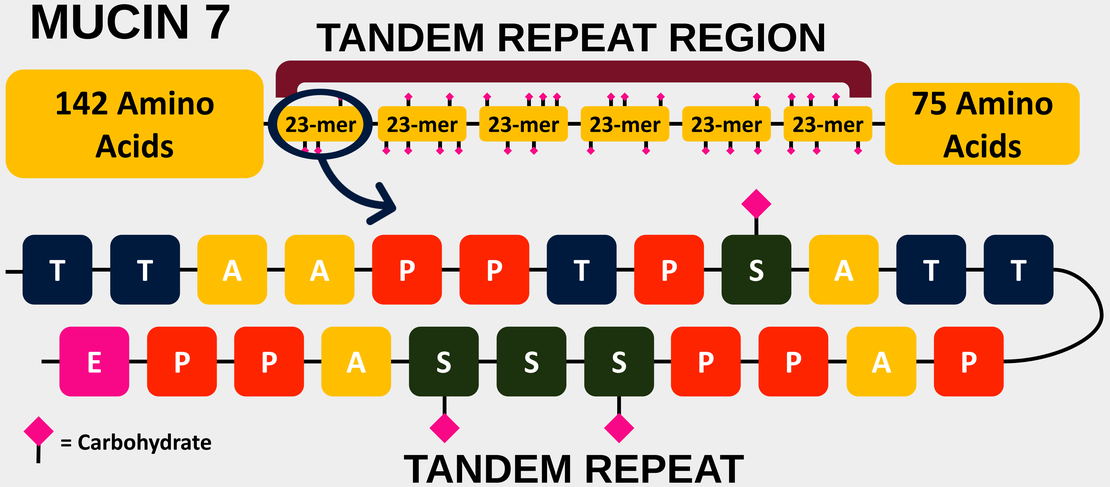
[1]North Idaho College, [2]Peptidaho Research Consortium, [3]University Of Idaho

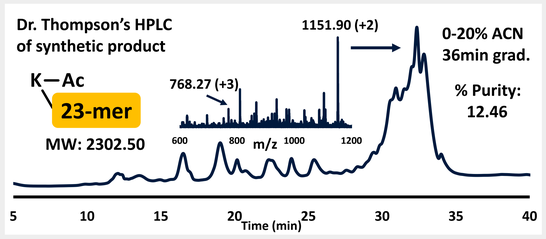
ABSTRACT

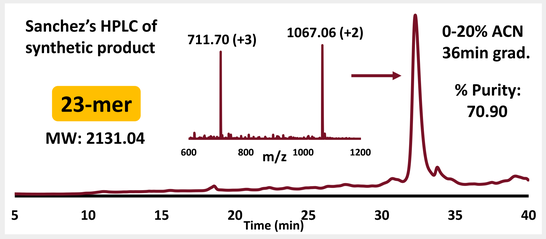
Mucin 7 is a salivary protein which has enigmatic functions. It is composed of 355 amino acids which include six tandem repeats rich in the target of O-linked glycosylation, threonine, and serine amino acids. There is evidence that Mucin 7 interacts with oral pathogens such as *Streptococcus gordonii,* yet Mucin 7's method of bacterial interaction and biological role remain uncertain[1]. Using Solid Phase Peptide Synthesis (SPPS), a tandem repeat (23-mer) without glycosylation can be easily synthesized to act as a control for further research. However, without a properly synthesized 23-mer, the synthesis of future 23-mers with C-linked glycosylations will likely be problematic. Thus, it is important to improve the current SPPS process. Another goal is to attach a fluorophore on the N-terminus of 23-mer for biological testing. Synthetic products were purified using RP-HPLC and correctly identified through ESI-MS. By changing the coupling time and confirming all reagents were uncontaminated, we achieved a higher purity synthesis of the 23-mer. Numerous experiments were conducted which reveals NBD as a potential fluorophore for biological testing. This project is now ready for the next phase: incorporating a C-linked glycosylated serine mimic into a new 23-mer[2].



MUCIN 7







Dr. Thompson's synthetic product

- Coupling time was **5 minutes**
- Multiple side reactions and deletions were observed
- Although the right peptide was successfully synthesized, improvement must be done to maximize purity of synthetic product
- To help with ionization, acetyl-lysine was attached

Sanchez's synthetic product

- Impure reagents were taken into consideration so new 20% piperidine and HBTU solutions were prepared
- Coupling time was changed to **10 minutes** to make sure the activated amino acid has enough time to couple with the polypeptide chain
- In case of a threonine to threonine coupling, the coupling time was increased to **20 minutes** because of steric hindrance

Result

- The synthesis of 23-mer was improved
- ESI-MS verified the mass of products and attachment of acetyl-lysine was not needed because the 23-mer ionized sufficiently

Control	Time (min) 0-40% ACN 26 min grad.	mH+ Calc. (g/mol)	mH+ Obs. (g/mol)	Yield
LYRAG	12.92	578.7	578.3	84%
YGGFM MET-enkephalin	19.90	573.7	573.2	98%

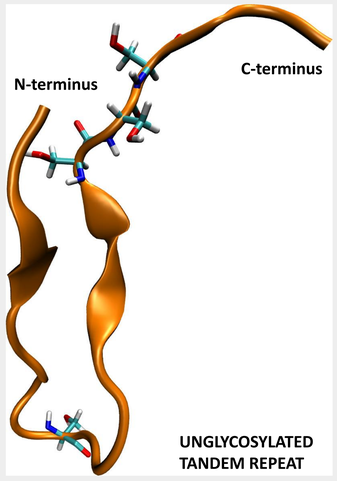


Figure 1: Predicted 3-D structure of tandem repeat with long MD simulations. The cyan colored sticks represent the serine residues.

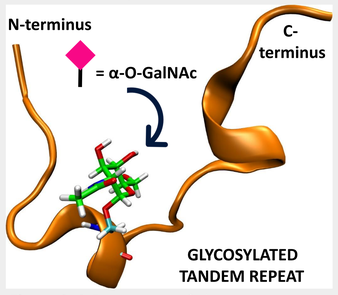
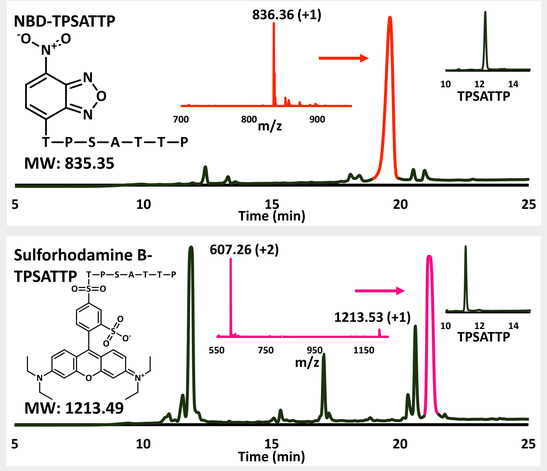


Figure 2: 3-D structure of glycosylated serine tandem repeat predicted by long MD simulations. The glycosylation changed the secondary structure from beta-sheets to helical form. The cyan colored stick represents serine residue while green colored sticks represent sugar moiety attached through O-linked glycosylation.



NBD vs Sulforhodamine B

- Incorporating a fluorescent molecule on the N-terminus of 23-mer makes it easy to see how it interacts with bacteria
- Polymer TPSATTP was synthesized to mimic the threonine ending of the 23-mer
- Multiple experiments were conducted to see the optimal condition to attach both fluorophores

	NBD	Sulforhodamine B
Mass	30 mg	30 mg
DIEA	20 µL	20 µL
Coupling time	24hrs	24hrs
Gradient	0-40% ACN 21min grad.	0-60% ACN 21min grad.

Result

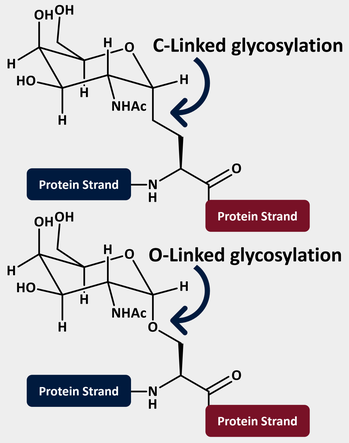
- Both fluorophores were successfully attached according to the ESI-MS result
- NBD is a better fluorophore compared to Sulforhodamine B
- Large peak of unreacted TPSATTP was present in Sulforhodamine B HPLC contrary to NBD HPLC
- Much less side reactions in NBD experiment compared to Sulforhodamine B experiment

Conclusion

- The synthesis of 23-mer was improved by changing coupling time and making sure reagents were not contaminated
- NBD was experimentally a better fluorophore than Sulforhodamine B
  - Complete peptide reaction
  - Much less side reactions observed
  - Cheap ($20/gram vs $323/gram)

Future Work

- New 23-mer will be synthesized with C-linked glycosylated serine mimics
- Because of its stability, O-linked glycosylation will be changed to C-linked glycosylation
- The 23-mer with *n* glycosylations (*n* = 0 - 4) will be assayed for interaction with bacteria to see if 23-mer of Mucin 7 is the region that binds with bacteria



Works Cited

- [1] Kesimer, M.; Kilic, N.; Mehrotra, R.; Thornton, D. J.; Sheehan, J. K., Identification of salivary mucin MUC7 binding proteins from Streptococcus gordonii. *Bmc Microbiol* **2009,** *9*.
- [2] Nolen, E. G.; Li, L. Y.; Waynant, K. V., Synthesis of beta-C-GlcNAc Ser from beta-C-Glc Ser. *J Org Chem* **2013,** *78* (13), 6798-6801.
- [3] Chan, Weng C, and White, Peter D. Fmoc Solid Phase Peptide Synthesis. **2000**.

Acknowledgment

- The project described was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grant #P20GM103408.
- Thanks to Gonzaga University for the use of ESI-MS under NSF grant CRIF:MU grant #0741868.
- The molecular modeling work was supported by the CMCI sponsored by the NIGMS under award number NIH P20 GM104420, through MAG to KW & DT.
- Special thanks to Rhena Cooper, Kirsten Blanchette, and every NIC staff who supported the NIC INBRE students throughout the summer.