# Characterization of an Eleven Residue MUC-1 Peptide Structure in solution by 2D NMR Spectroscopy

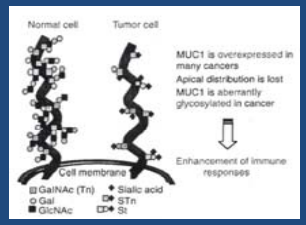
Michael McAnally (Dr. Thao Yang), Department of Chemistry, University of Wisconsin-Eau Claire

## Abstract:

MUC-1 peptides are peptides based on the amino acid sequence of the tandem repeat domain of mucin expressed by cancer cells, which immunity in a host could be induced against. MUC-1 peptides have been used as antigenic agents in the development of cancer vaccine. The purpose of this research is to better understand the specific conformation of a specific MUC-1 peptide. To address the question, we have synthesized an 11-mer peptide with the sequence GVTSAPDTRPA that spans the main portion of the tandem repeat domain of mucin that is known to bind to MUC-1 monoclonal antibody. The presentation will include data on the characterization of the MUC-1 peptide by preparative HPLC, LC-MS, 2D NMR, and computational modeling of the peptide. Results suggests that there exists a β turn localized along the Ala5-Thr8 residues on the peptide backbone in solution.

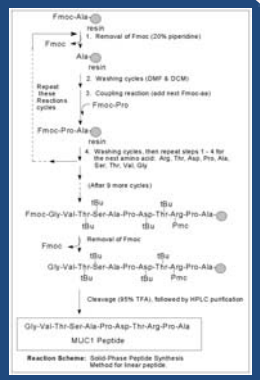
## Introduction:

Mucous consists of many different glycoproteins, attached to these proteins are carbohydrate chains of varying lengths. A family of these glycoproteins from the mucous that are encoded by the MUC-1 gene are known as mucins (or MUC-1 mucins). MUC-1 mucins are expressed by many types of epithelial cells within the human body, including tissues of the breast, lung, prostate, pancreas, ovaries, and testes. One of the functions of normal mucins is thought to be protection of the cell surface; however, when epithelial cells become cancerous, the mucins are overexpressed and under-glycosylated compared to those from healthy cells. It has been established that the change (i.e., under-glycosylation) on the surface of mucin is capable of inducing immune response in patients with cancers. Such immune response is thought to be caused by certain region or segment of the mucin structure. One of these segments on the mucin is believed to be the Tandem Repeat Domain of the mucin structure, which consists of the 20-amino acid sequence, Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala-His.



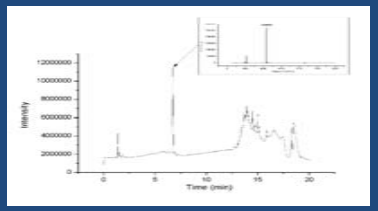

## Synthesis:

Synthesis of the MUC-1 peptide was carried out manually by FMOC-chemistry, using the Solid-Phase Peptide Synthesis technique. The synthesis began with a solid resin (Wang Resin), from which the peptide chain is lengthened by coupling with subsequent FMOC-amino acids that formed the sequence of the peptide. Once the desired sequence is obtained, the peptide chain is cleaved off from the resin by 95% TFA, leaving the desired crude product in solution, from which HPLC was used to isolate and purify the desired peptide (11-mer, MUC-1 peptide).



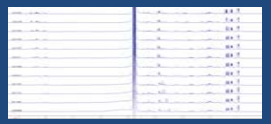

## Purification:

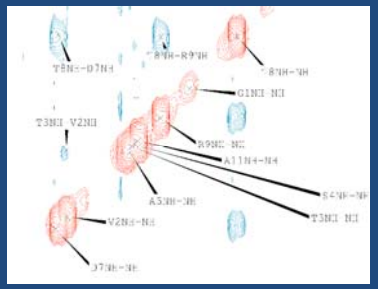
Purification of the MUC-1 peptide was done using a preparative HPLC (High Performance Liquid Chromatography), in which it was possible to isolate the peptide from impurities present. HPLC purified MUC-1 peptide was analyzed on the Agilent 6210 Time-of-Flight LC-MS Spectrometer. Here the peptide was passed through a $C_{18}$ column, then analyzed by the Mass Spectrometer by the Electro-Spray Ionization (ESI) technique. The chromatogram shows the Total Ion Current (TIC) identical to the traditional HPLC chromatogram in intensity versus retention time. The indicated peak contained the 11-mer MUC-1 peptide, and the inset graph shows the mass spectrum of the MUC-1 peptide at a mass of 1069.54 (m/z) compare to a theoretical mass of the 1070.17 (m/z) (in negative mode).




## NMR Studies:

Utilizing a 400MHz Bruker-Avance NMR spectrometer it was possible to run Total Correlation Spectroscopy (TOCSY) which shows the interaction of two $^1$H atoms within 3 bonds of each other, Rotating Frame Overhause Effect Spectroscopy (ROESY) an experiment that shows distance between protons that are in close proximity in space to each other, and Nuclear Overhauser Enhancement Spectroscopy (NOESY) experiments, which typically show the same data as a ROESY, but can at times provided new data, as well as HSQC, in numerous solvents including a phosphate buffer, a phosphate buffer with micelles, and dimethyl sulfoxide (DMSO). 1D NMR data were studied as a function of pH and temperature were also examined.





ROE of the NH-NH$_i$

## NMR Results:

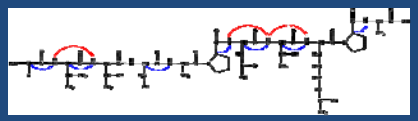
When examining the NMR data many consistent ROE are observed which give some interesting leads to the structure:

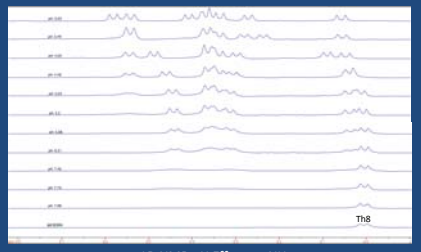
- The backbone between Asp7-Arg9 is relatively compact
- The backbone between Val2-Ala5 appears to be in a compact structure

From the pH and temperature variation studies, it was observed that:

- Gly1 NH is rapidly exchanging with solvent
- Thr8 NH is hydrogen bonded

The ROEs appear to indicate a β turn along the Ala5-Thr8 residues as it follows referenced ROE patterns

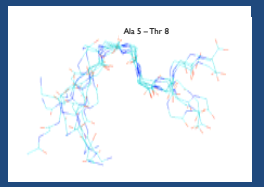


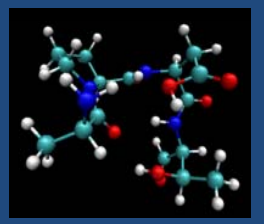



1D NMR pH Effect on NH

## Structure Calculations:

Using the program XPLOR-NIH (Version 2.19) the following structures were calculated from inputs of the ROE and NOE observed. Note that there are numerous calculated structures with similar structure along the Ala5-Thr8 residues and the sample of the observed turn from one of the calculated structures.



## Conclusions:

- The 11-mer linear mucin peptide with the sequence GVTSAPDTRPA contains a β turn localized on the Ala5-Thr8 residues.
- Structure of the turn appears to be consistent given the negligible change in ROE observed in different environments.

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## Acknowledgements:

- Department of Chemistry, UW-Eau Claire
- UW-Eau Claire Office of Research and Sponsored Programs (ORSP)
- Ronald E. McNair Postbaccalaureate Achievement Program
- Mr. Daniel H. Rose, for collaboration on 2D NMR processing and analysis, as well as preparative HPLC.
- Ms. Theresa Swanson, for collaboration on synthesis of the peptide.