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Fast Photochemical Oxidation and Footprinting of Proteins Via Trifluoromethyl Radical Chemistry

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FAST PHOTOCHEMICAL OXIDATION AND FOOTPRINTING OF PROTEINS VIA TRIFLUOROMETHYL RADICAL CHEMISTRY

By

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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College and a Bachelor of Arts in Chemistry from the Department of Chemistry at the University of Mississippi.

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Reader: Professor Emily Rowland

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Reader: Professor Paul Boudreau

DEDICATION

This thesis is dedicated to my family. Thank you for the love and support and the endless compassion. I love you all.

ACKNOWLEDGEMENTS

This page is to thank those who have made the completion of this thesis possible. To Dr. Sharp, thank you so much for believing in sophomore me to complete your work and research. Without your assistance and guidance, I would not have been able to continue my journey smoothly and elevate my knowledge of chemistry and biochemistry. Your encouragement made the 8-hour days in the laser lab worthwhile. To Dr. Sandeep Misra, thank you for teaching me the importance of mass spectrometry and how to operate all my machinery. It was your tireless help and willingness to answer my questions that helped me along the way to perform these experiments and I truly could not have done it without you. I would like to thank the Sharp Lab group, Selina Cheng, Anter Shami, Dr. Suman Choudhary, Darrienne Martin, Sidney Stuckett, and Lyle Tobin for being a wonderful group to work alongside and with. Without you all, I would have not learned valuable lessons and how to be a better scientist, so thank you deeply for everything.

ABSTRACT

ELAINE MORROW:

(Under the direction of Dr. Joshua S. Sharp)

Fast photochemical oxidation of proteins (FPOP) is a useful tool in proteomics because of the ability for modifications to occur on the scale of microseconds which reduces the modifications to tertiary and quaternary structure allowing for more accurate labeling of the protein. Labels for FPOP are generated from various radicals in our experiments which include hydroxyl radicals and trifluoromethyl radicals. Hydroxyl radicals are easily generated by using an excimer laser (KrF laser, 248 nm) or a UV flash lamp (as a part of the Fox™ System) by the photolysis of hydrogen peroxide. Trifluoromethyl radicals, however, need hydroxyl radicals to be generated because at this time, a suitable photolytic precursor compound has not been found to photolyze in the proper conditions need during standard FPOP.

Trifluoromethyl radicals are desired in footprinting because it can label 18 out of the 20 common amino acids as well as it could be used to label glycoproteins or carbohydrate because it does not break apart the carbon sugar rings like hydroxyl radicals do. The generation of trifluoromethyl radicals under these conditions will allow for easier footprinting of complex mixtures of carbohydrates and proteins without sacrificing structural integrity. By having a useful way to mark both R-groups on amino acids and carbohydrates, this can be used to develop

proteomics for different pharmaceuticals and proteins within the body and widen our knowledge in protein-drug interactions.

Radical dosimetry is an extremely useful tool in FPOP and can give the user a helpful approximation of oxidation of the protein in question. Experiments on pyromellitic acid (PMA) were performed to see if it was a suitable candidate compound for dosimetry by fluorometry. Because of the chemical's composition, price, and ease of use, it seemed like a prime candidate for inline fluorometry dosimetry with FPOP. Experiments and data showed an unknown reaction that showed the chemical fluorescence activity after exposure to the laser without an oxidant present.

PREFACE

This thesis work was a continuation of the projects previously performed by Addison Roush. After my graduation, I hope to pass my work onto my mentee, Lyle Tobin, to keep the spirit of this project alive.

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LIST OF ABBREVIATIONS

BPCA	Benzenepentacarboxylic acid
BSA	bovine serum albumin
CID	Collision induced dissociation
DTT	Dithiothreitol
ETD	Electron transfer dissociation
FPOC	Fast photochemical oxidation of carbohydrates
FPOP	Fast photochemical oxidation of proteins/peptides
LC-MS/MS	Liquid chromatography coupled to tandem mass spectrometry
NaTF	Sodium Triflinate ($NaSO_2CF_3$)
NaTFA	Sodium Trifluoroacetate ($NaCO_2CF_3$)

Chapter 1: Fast Photochemical Chemistry

I. Fast Photochemical Oxidation of Proteins (FPOP) with Hydroxyl Radicals (•OH):

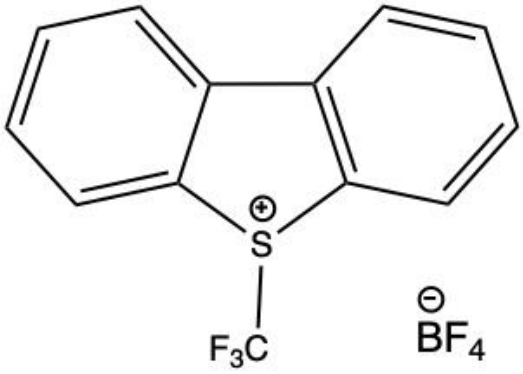
The oxidation of proteins by means of free radicals have been well studied in the fields of biochemistry due to the irreversible modifications done to amino acids and genomes.[1,2] Because of the irreversible nature of these modifications, free radical addition can be a useful tool in proteomics showing which residues have access to the surrounding solvent.[3] FPOP using the photolysis of hydrogen peroxide has been well studied and proven to be effective in modeling different protein and ligand interactions. FPOP with hydroxyl radicals is desired when using proteins due to many reasons. Hydrogen peroxide can easily photolyze into the radicals. Experiments are also cost effective and relatively green. There are no additional steps to FPOP with hydroxyl radicals which keeps protein concentrations high for analysis and allows for quick analysis. While the premise of the experiment has not faltered, the method in which hydroxyl radicals have been. X-Ray photolysis of water, KrF excimer laser, and the Fox™ Photolysis System are all proven instruments that have been used in the generation of hydroxyl radical and each one is increasingly safer and less material intensive than the last.[4,5,6] Each method relies on fast chemical processes that generate hydroxyl radicals that modify the protein irreversibly and before conformational changes can occur. Although some reactions continue to happen in the solution, these are controlled by quenching the solutions and isolating them from the collective data.[8] While an extremely useful tool when in use with liquid chromatography and tandem mass spectrometry (LC-MS/MS), its application is not useful for carbohydrates or glycoproteins. This

is because the hydroxyl radical can break apart the sugar ring creating complex products which are extremely hard, or impossible, to correctly identify reactive surfaces. Because of the complex chemistry involved, this process limits biological glycoproteins and ligands which are extremely important in pharmacological and immunological applications.[8] The goal to properly achieve accurate proteomic data of glycoproteins and carbohydrates lies with a different radical that can interact with both sugars and amino acid chains to provide a marked product that can be easily analyzed.[9]

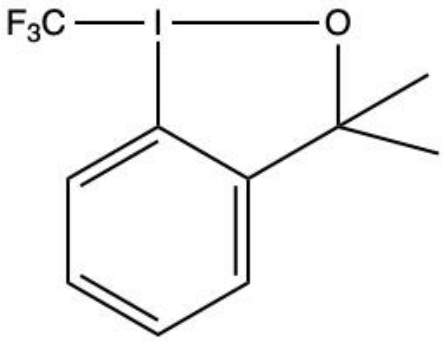
II. Fast Photochemical Oxidation of Proteins and Carbohydrates with Trifluoromethyl Radicals (•CF_3):

Trifluoromethyl (-CF_3) groups are present in many pharmaceuticals in the market today and have many practical uses such as antibacterial, corticosteroids, and many other uses.[1, 10] Beside the use in pharmaceuticals, the radical form (•CF_3) is also used in proteomics to mark proteins and carbohydrates. The radical also has a higher affinity for some amino acids that have a low reactivity or are silent with the hydroxyl radical such as glycine, alanine, and serine. The trifluoromethyl radical readily reacts with 18 out of the 20 common amino acids. Although a perfect candidate for the desired mechanism of attachment, the radical comes with some set back in the ways it is normally generated. Many reagents such as Umemoto's reagent, Togni's reagent, and CF_3I are either not soluble in water, which is needed due to the proteins not being stable in other solvents, or only available in gaseous forms which is not desirable to work with.[11] Reagent compounds can be seen in **Figure 1.** Other chemicals that contain -CF_3 are available in water soluble compounds, but they cannot be photolyzed to produce the radical needed to footprint the protein. Some compounds do follow the photolysis and water-soluble requirements but require expensive or not

commercially available metal-organic catalysts that cannot be separated out or require many steps that reduce the yield of the labeled protein significantly. The research work I have done aimed to find a suitable candidate chemical that can be used in biologically compatible solutions without the need for catalysts. At this time, a combination of Langios's reagent and hydrogen peroxide is needed to create the trifluoromethyl radical. This method is not desired for footprinting carbohydrates or glycoproteins because both hydroxyl radical footprinting and trifluoromethyl radical footprint occur.



Umemoto's Reagent 1



Togni's Reagent 1

Figure 1: Umemoto's and Togni's Reagent. Pictures created in ChemDraw 20.0

III. FPOP/FPOC Workflow:

The workflow of FPOP is reliant on the system of photolysis. In our lab, the Fox™ System is currently used. To start FPOP, the running buffer must be prepared and flushed through the system. In experiment performed and later discussed in this work, a 20 mM concentration of sodium phosphate buffer is used. For the solution that is directly injected into a loop and pushed through the system is then prepared. For the experiment performed, it consists of a mix of protein/carbohydrates/peptide (final concentration ranges from 5.0 uM to 25 uM), the trifluoromethyl containing chemical (either sodium trifluoroacetate or sodium triflinate), and hydrogen peroxide, which is added immediately before injection. By adding the hydrogen peroxide at the last minute, it reduces the modification due to oxidative stress and reduces chances of protein unfolding. This mixture is injected and pushed through the plastic injection loop to a 250 μm ID fused silica capillary with a UV-transparent coating as to all the UV rays to photolysis the sample[6]. The mixture then arrives at the flash lamp window. This lamp operates by flashing high voltage UV light. The flashing mechanism allows the solution to be exposed to light once and reduce multiple oxidations or modifications and has an exclusion volume due to the flow rate and flashing of the lamp. Double exposure of the proteins not only increases modifications but can unfold the protein making the analysis inaccurate and makes the footprint useless for proteomics. The sample passes through the capillary to the UV absorbance lamp. It then passes through and is collected in a microcentrifuge tube which contains a quenching solution of catalyst and methionine amide for peptides and proteins and just methionine amide for carbohydrates. This solution quenches any excess radicals, secondary oxides created, and the excess hydrogen peroxide. Modified proteins are then digested using trypsin to help with analysis by (LC-MS/MS) and further bottom-up proteomic analysis. This workflow is shown graphically in **Figure 2.**

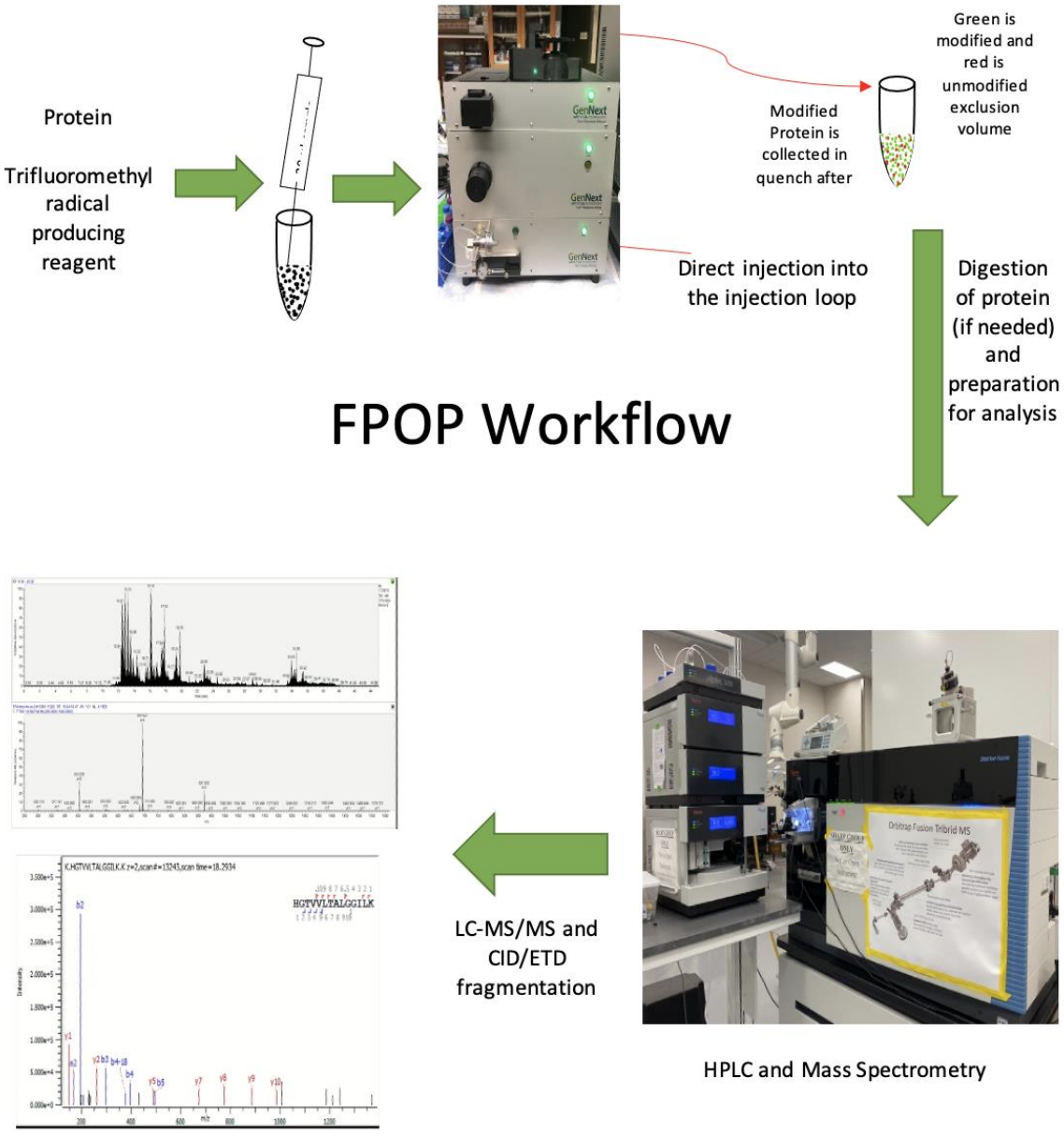


Figure 2: Fast Photochemical Oxidation of Proteins or Carbohydrates Workflow

IV. Dosimetry for FPOP:

Radical dosimeters are chemicals that are added to the FPOP mixture that are modified along with the protein, where the modified dosimeter has an easily measured difference in properties such as UV absorbance or fluorescence. These radical dosimeters can be used to estimate the effective dosage of hydroxyl radicals created and scavenged during the experiment. These chemicals included adenine and tris(hydroxymethyl)aminomethane[6,11]. By using an inline UV absorbance spectrometer, the chemicals give a reading of the UV absorbance of the solution. Inline dosimetry allows real-time modifications to the experiment to ensure that samples are irradiated with the same energy and concentration of radicals to help maintain consistent oxidation throughout the experiment. In the research performed early in my lab experience, pyromellitic acid (PMA) and benezepentacarboxylic acid (BPCA) was tested as an effective dosimeter for hydroxyl radical concentration. Rather than using UV absorbance with PMA and BPCA, an inline fluorometer can be used. Since the unoxidized forms do not fluoresce, in theory, the chemicals could be added before the experiment and the fluorescence intensity could be used as a dosimeter for the hydroxyl radicals. However, these chemicals reacted with the laser in absence of the hydroxyl radical and had fluorescence activity making them poor candidates for inline fluorescence dosimeters. The supporting data can be found in **Figures 3 and 4.** The two chemicals did not have a linear growth in response to the hydroxyl radical concentration, so the use as a dosimeter could lead to an incorrect calculation of radical dosage. This method would also require an inline fluorometer to ensure that decay of the product does not occur. For dosimetry, it is important to have a linear growth so that hydroxyl radical production could be measure as well as having a chemical that does not increase fluorescence without the presence of hydroxyl radicals as seen in **Figure 3.**

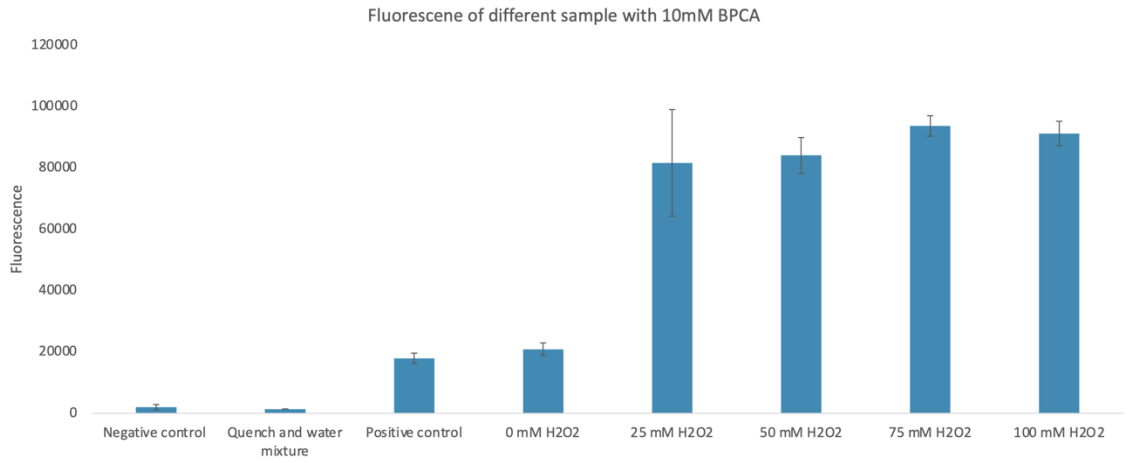


Figure 3: Fluorescence of BPCA with various H_2O_2 concentrations. This chart shows nonsignificant increase in fluorescence with H_2O_2 concentrations as well as a high background reading at 0mM.

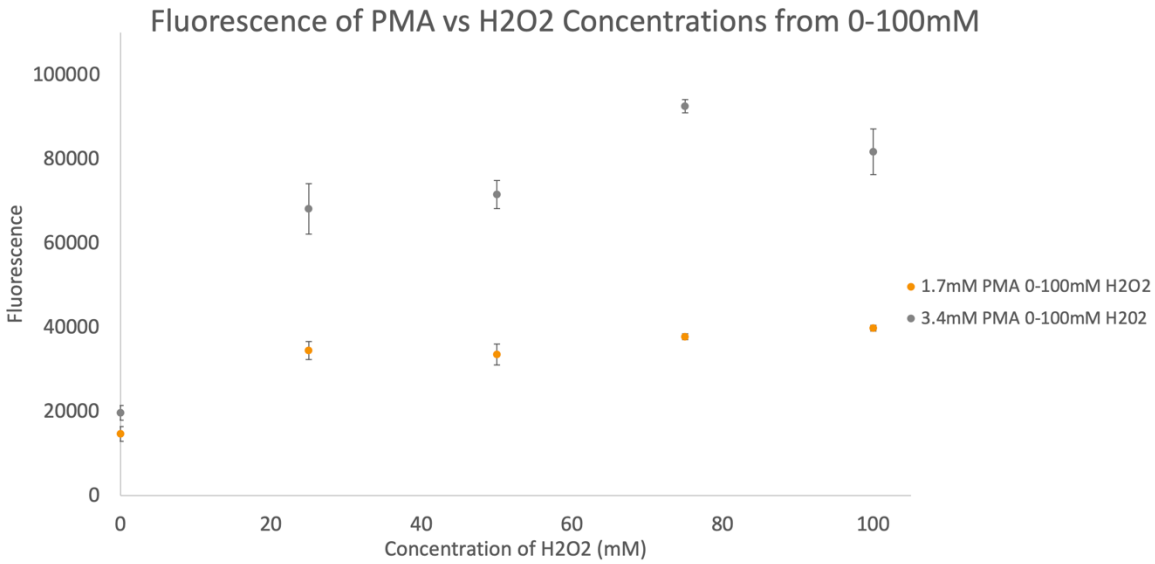


Figure 4: Fluorescence of PMA with various H_2O_2 concentrations. This figure shows a nonlinear growth of signal as well as a high level of background fluorescence after laser treatment.

Chapter 2: Sodium Trifluoroacetate and Sodium Triflinate Experiments

I. Materials and Methods

All reagents that were used in the experiments were of the highest quality available and were not additionally purified. Myoglobin from equine skeletal muscle, human Glu[1]-fibrinopeptide B (GluB), Catalase from bovine liver, and sodium triflinate were purchased from Millipore-Sigma Corporation (St. Louis, MO). Sodium phosphate monobasic, sodium phosphate dibasic, and sodium trifluoroacetate (NaTFA), LC/MS-grade water, LC/MS-grade formic acid, and LC/MS-grade acetonitrile were purchased from Thermo-Fisher Scientific Corporation (Waltham, MA). Methionine amide was purchased from Bachem (Torrance, CA). Sequence grade modified trypsin was purchased from Promega Corporation (Madison, WI).

In the proposed reaction, sodium triflinate along with low concentrations of hydrogen peroxide could create the trifluoromethyl radical which then binds to the protein or carbohydrate. To create the radical, the Flash Oxidation (Fox™) Protein Footprinting System used a high energy plasma photolysis source to break apart the chemical (either NaTFA or hydrogen peroxide)[1]. Inline dosimetry was not used in any experiment preformed because adenine is not proposed to react with trifluoromethyl radical to change absorbance and therefore will not act as a good dosimeter. Based on UV absorbance and emission given by a Thermo NanoDrop 2000c UV spectrophotometer, the absorbance of NaTFA and sodium triflinate solutions are around 220 nm and 260 nm respectfully. After flashing, 12 uL exposed protein samples (GluB and myoglobin)

were collected in a 15 uL of quench solution composed of 0.3 mg/ml catalase and 35 mM methionine amide to reduce secondary oxidation or trifluoromethylated products and to digest the residual hydrogen peroxide. For 12 uL exposed carbohydrate samples, a 15 uL quench solution of 35 mM of methionine amide was used so that digestion was not needed. 1.0 M Tris pH 8.0 and 100 mM DTT were added to final concentrations of 50 mM and 5 mM respectfully. The collected protein samples and were incubated at 100 °C for 30 minutes to denature and to break apart the disulfide bridge created by cysteine-cysteine bonding. After the denaturing is complete, the samples were allowed to cool to room temperature for at least 15 minutes. A 1:20 w/w ratio of trypsin to protein was added to the mixture and placed in a rotating warmer set at 37 °C. The sample were allowed to react overnight so that the trypsin could digest the samples into smaller peptide to make LC/MS analysis simpler. Digestion was halted by adding 0.1% formic acid. The samples were then transferred into vials and placed in the LC storage for further analysis. For LC/MS analysis, a Thermo Fisher Scientific Ultimate 3000 HPLC machine was used in tandem with the Thermo Fisher Orbitrap Fusion Tribrid mass spectrometer was used.

For LC/MS-MS analysis, the fragmented myoglobin segments were separated by an Acclaim PepMap 100 C18 nanocolumn (0.75 mm x 150 mm, 2um, Thermo Fisher Scientific). A forty-five-minute method and gradient was used for analysis. From 0 to 3 minutes, there was a steady flow of 98% solvent A and 2% solvent B. Solvent A was composed of 0.1% formic acid in LC/MS grade water, and solvent B was composed of 0.1% formic acid in LC/MS grade acetonitrile. Solvent B was then steadily ramped to 32% from 3 minutes to 30 minutes. Solvent B was then ramped to 95% in 1 minute. The 95% solvent B and 5% solvent A was held constant for 4 minutes. Solvent B was return to 2% over the course of a minute. The 98% solvent A and 2% solvent B was then held constant for 9 minutes. After this process was completed, another sample

or blank was run. The samples were preceded by two blanks and a quality check of BSA. [12] The visual representation of the gradient can be seen in **Figure 5.**

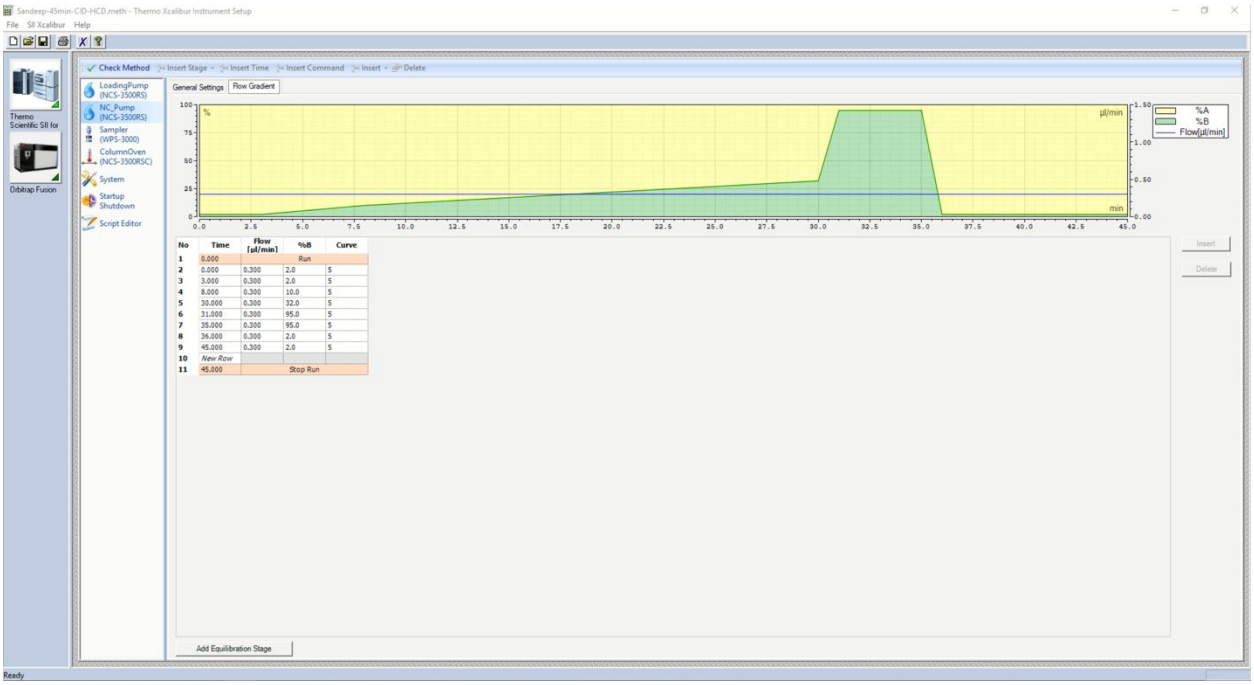


Figure 5: Visual gradient of HPLC flow and rate over the 45-minute run

The method used in Sharp et. al was used to calculate oxidation and trifluoromethylation events per peptide.[6] Byonic software version v3.9.4 (Protein Metrics, San Carlos, CA) was used to first identify modified peptides and to see the sequence coverage given by the scan. The masses of those peptides with and without modifications were then isolated on an ion chromatogram, and the correlated peak of those masses were then integrated. The values of those integrated peaks of modified peptides, $(+16)_{ox}$, $(+32)_{ox}$, $(+48)_{ox}$, or $(+68)_{TFM}$, $(+136)_{TFM}$, and $(+204)_{TFM,}$ were then used in **Equation 1** to calculate the events per peptide where M is the mass of the radical added and I is the peptide. The mass shifts add the mass of the different radicals, either hydroxyl radical or trifluoromethyl radical, while subtracting the mass of a hydrogen which is taken off after the addition. In the equation, the different additions are weighted by the number of reactions the

residue would have to undergo to add 2 or 3 additions. Double and triple oxidization or trifluoromethylation events are going to be weighted more than single addition events.

$$n_{mod} = \frac{[I(+1M)_{mod}\, x1 + (+2M)_{mod} x2 + I(+3M)_{mod} x3]}{[I_{unmod} + I(+1M)_{mod} + I(+2M)_{mod} + I(+3M)_{mod}]}$$

Equation 1: Calculations for the average modification seen on a peptide

II. Results and Discussion

In our experiment, we used the generation of hydroxyl radicals to successfully produce trifluoromethyl radicals and subsequently created trifluoromethylated products. After running digestion and mass spectrometry on the samples collected after experimentation, the sequence coverage was determined from Byonic software. This software takes the masses and the sequence of the protein and identify fragment of the peptide and assigns them a score of the quality of the peptide-spectrum math on a scale of 0-1000 where 1000 is the best score a peptide can receive[13]. The coverage from both proteins were 100% meaning that all residues were represented in the residues present after mass spectrometry and the isolated peptide scores under 250 were excluded when looking at possible peptides for analysis but remain in the coverage. This means that although some peptides that resulted in the sequence coverage, that if they scored less than 250, the peptides were excluded from the analysis. These peptide coverages which also includes peptide abundance or intensity can be seen in **Figures 6 and 7.**

Prot. Rank	Protein Name	|Log Prob|	Best |Log Prob|	Best Score	# Spectra	# Uniq. Peps.	# Mod Peps.	% Cov.	# AAs	Intensity
1 | 1	>sp|P68082|MYG_HORSE Myoglobin OS=Equus caballus GN=MB PE=1 SV=2	217.76	13.02	845.2	603	77	54	100.0	154	2.411e+9
2 | 2	>Reverse >sp|P68082|MYG_HORSE Myoglobin OS=Equus caballus GN=MB PE=1 SV=2	0.01	1.36	341.3	38	21	15	65.6	154	1.286e+7

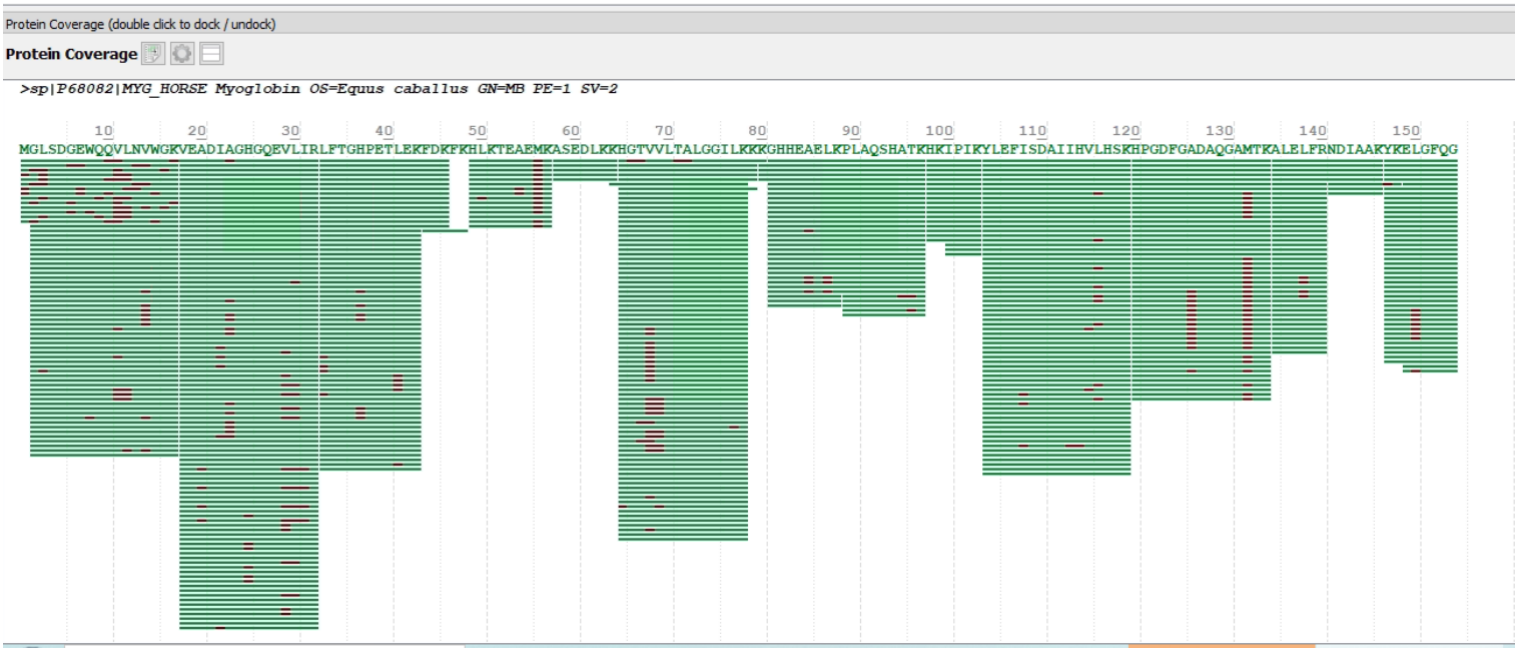


Figure 6: Peptide coverage and intensity of Sample 1(100% coverage with an intensity of 2.41×10^9)

Prot. Rank	Protein Name	|Log Prob|	Best |Log Prob|	Best Score	# Spectra	# Uniq. Peps.	# Mod Peps.	% Cov.	# AAs	Intensity
1 | 1	>sp|P68082|MYG_HORSE Myoglobin OS=Equus caballus GN=MB PE=1 SV=2	220.03	13.23	824.0	717	85	63	100.0	154	2.323e+9
2 | 2	>Reverse >sp|P68082|MYG_HORSE Myoglobin OS=Equus caballus GN=MB PE=1 SV=2	0.01	1.60	352.7	33	23	18	44.2	154	2.056e+7

Figure 7: Peptide coverage and intensity of Sample 2 (100% coverage with an intensity of 2.323 x 10^9)

The combination of that proved to have successful results was 40 mM sodium triflinate and 5 mM H_2O_2 in the running solution. This combination was the fourth combination tested after only sodium trifluoroacetate, only sodium triflinate, and sodium trifluoroacetate and hydrogen peroxide. This combination proved to have successful labeling on 37 H and can be seen in **Figure 8** on the MS/MS spectrum.

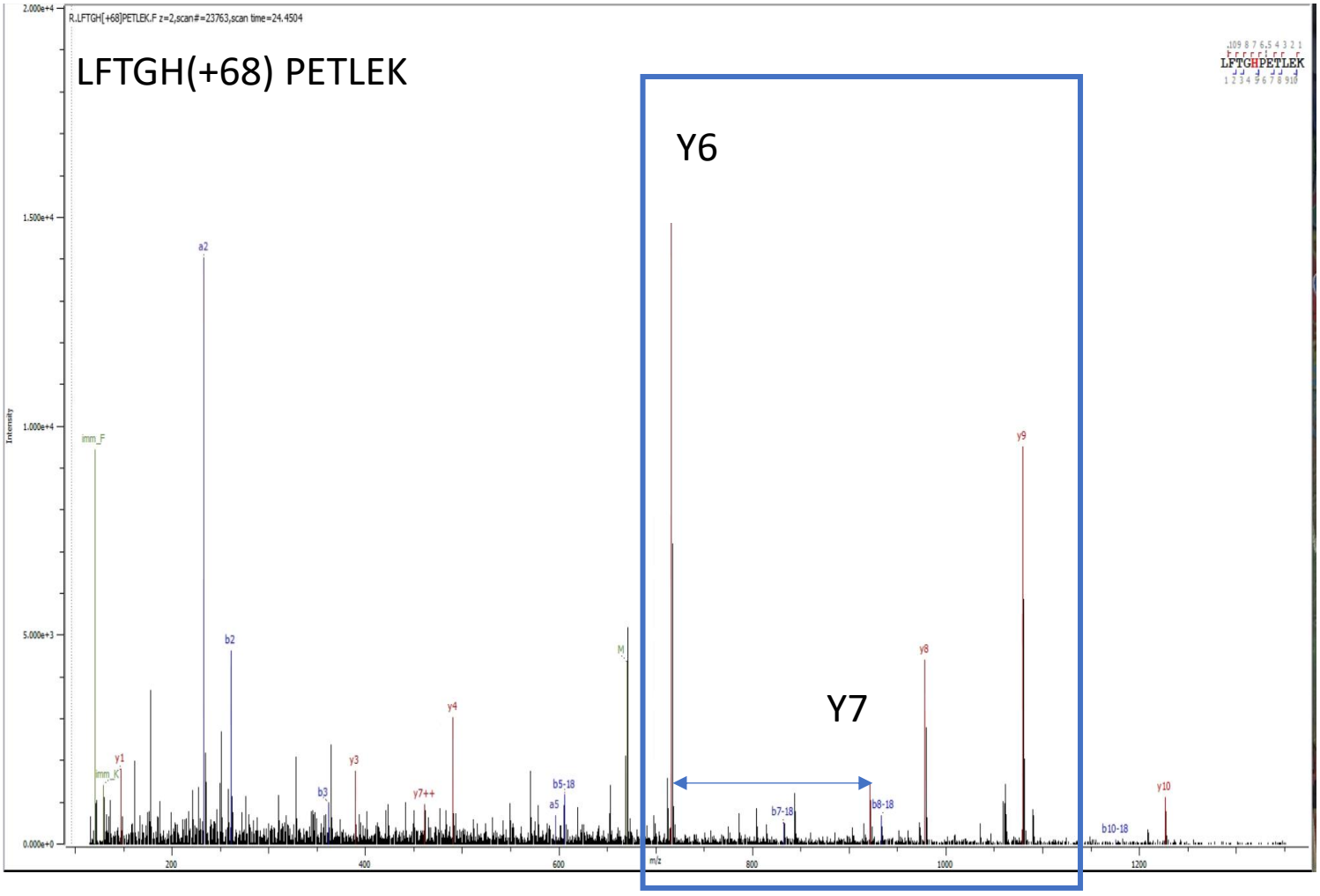


Figure 8: MS/MS spectrum of Peptide 33-44 showing trifluoromethylation on 37H.

This proves a mass shift of +34 which matches the charge state and mass shift expected on the segment. This mass shift and MS/MS spectrum was consistent between the two samples that underwent the successful method. The mass range spectrums can be seen in **Figure 9 and 10** for the unmodified segment of the protein and the modified segment of the protein. These mass ranges show a shift and equal charge states and proves that our product is visible in the sample.

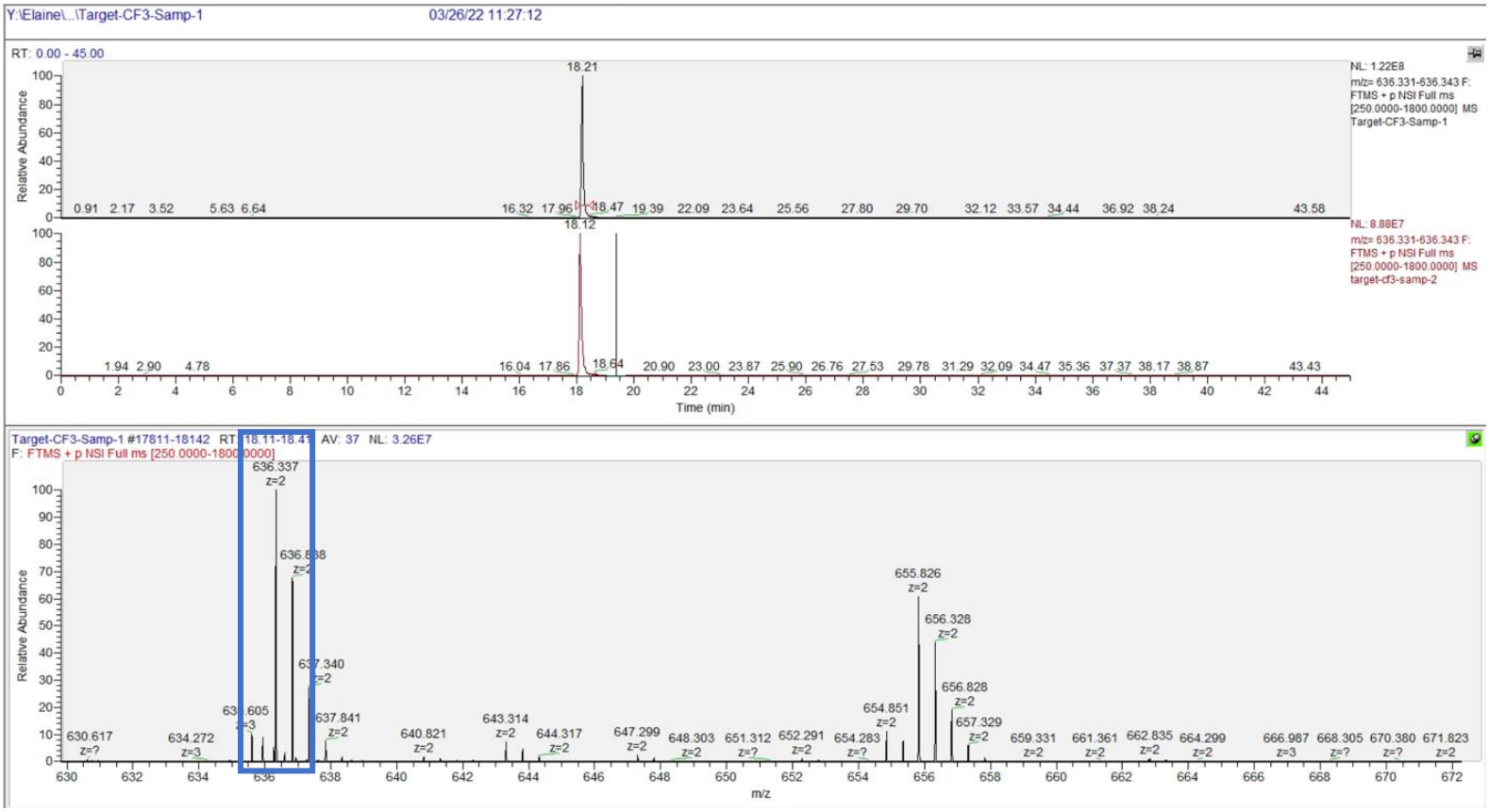


Figure 9: Mass range and MS of unmodified peptide 33-44 of both samples with a box around the target MS value for the peptide (636.337 Da, z= +2)

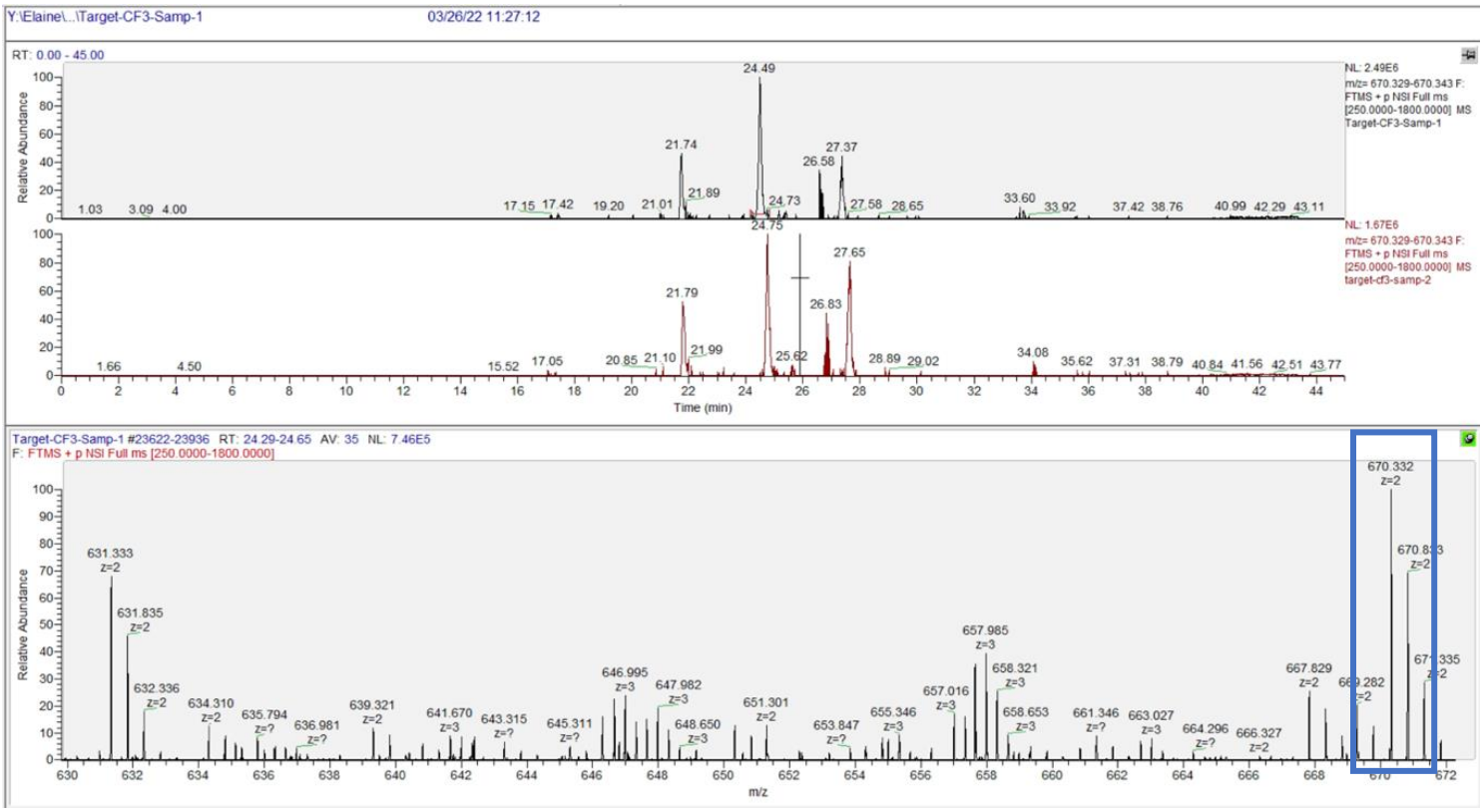


Figure 10: Mass range and MS of peak with modified peptide 33-44 of both samples with a box around the target MS value for the peptide shown in Figure 7(670.332 Da, z = +2)

This data clearly shows that the mass shift seen on the MS and the rendition shift on the mass range are consistent with trifluoromethylation of myoglobin. To calculate the average trifluoromethyl events, we use equation 1 and the integrated area underneath the peak in the mass range graph. By using the area underneath the curve, we get peptide segments that were retained at that time relative to the abundance of that peptide sequence. The average trifluoromethylation events for the first three peptide sequences can be found in **Figure 11.** This chart shows the percentage of events based on mass spectrometry analysis using the equation where 1 represents events were seen in 100% of the peptide with the same sequence.

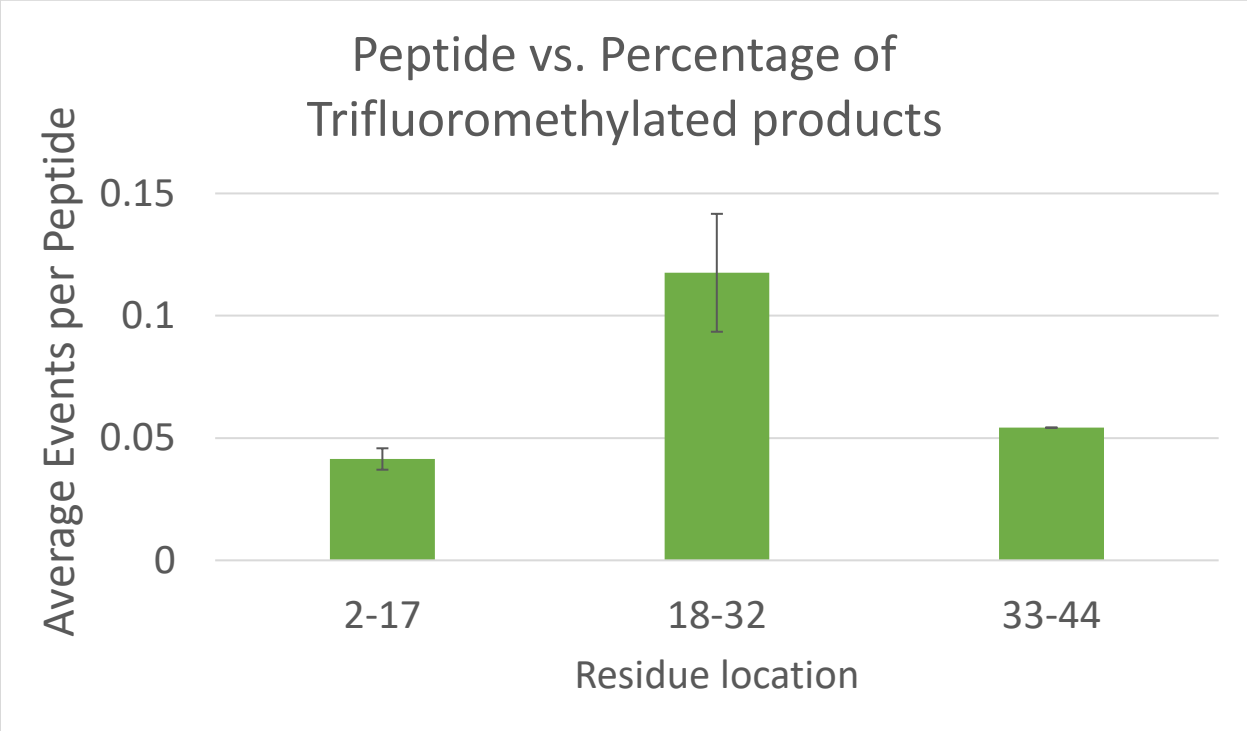


Figure 11: Average events of trifluoromethylation of peptides of myoglobin. Three peptides were detected and confirmed as modified. Error bars represent one standard deviation from a duplicate measurement.

In the future, this method will potentially be used to determine glycoproteins and sugar interactions with proteins because it is proposed that trifluoromethyl radical can interact with both sugars and proteins to provide simple, footprinted products. The hydroxyl radicals can attack both sugars and proteins, but they produce complex products and difficult results to analyze due to the likelihood that the sugar ring can be oxidized and opened. Glycoproteins and sugar-based compounds are easily oxidized by those free radicals which can open the sugar ring and cause complex and inaccurate compounds for analysis. For analysis that may be dependent on drug-glycoprotein

interaction, it is extremely important for sugars and glycoproteins to be in a native form so that accurate analysis can happen. Carbohydrates have been successfully trifluoromethylated through different photocatalyst[13], however, they use incompatible solvents for glycoproteins. Future projects conducted at GenNext Laboratories in California and Washington University could help to prove robustness and the reproducibility of the methods. Our lab is currently running a sample of NAG-3 sugar with the method, revised to have the maximum 120 flashes to increase percentage of modified products, which should hope to provide results of successful trifluoromethylation of sugars.

LIST OF REFERENCES:

1) Garrison, W. M. (1985). Reaction Mechanisms in the Radiolysis of Peptides, Polypeptides and Proteins. *American Chemical Society*, *1987*(87), 381–398. https://doi.org/10.2172/5415209
2) Sato, S., & Nakamura, H. (2019). Protein Chemical Labeling Using Biomimetic Radical Chemistry. *Molecules*, *24*(21), 3980. https://doi.org/10.3390/molecules24213980
3) Johnson, D. T., Di Stefano, L. H., & Jones, L. M. (2019). Fast Photochemical Oxidation of Proteins (FPOP): A Powerful Mass Spectrometry–based Structural Proteomics Tool. *Journal of Biological Chemistry*, *294*(32), 11969–11979. https://doi.org/10.1074/jbc.rev119.006218
4) Cheng, M., Asuru, A., Kiselar, J., Mathai, G., Chance, M. R., & Gross, M. L. (2020). Fast Protein Footprinting by X-ray Mediated Radical Trifluoromethylation. *Journal of the American Society for Mass Spectrometry*, *31*(5), 1019–1024. https://doi.org/10.1021/jasms.0c00085
5) Vahidi, S., & Konermann, L. (2016). Probing the time scale of FPOP (fast photochemical oxidation of proteins): Radical Reactions Extend over Tens of Milliseconds. *Journal of the American Society for Mass Spectrometry*, *27*(7), 1156–1164. https://doi.org/10.1007/s13361-016-1389-x
6) Sharp, J. S., Chea, E. E., Misra, S. K., Orlando, R., Popov, M., Egan, R. W., Holman, D., & Weinberger, S. R. (2021). Flash Oxidation (Fox) System: A Novel Laser-Free Fast Photochemical Oxidation Protein Footprinting Platform. *Journal of the American Society for Mass Spectrometry*, *2021*(32), 1601–1609. https://doi.org/10.1021/jasms.0c00471.s001

7) Finch, A., & Pillans, P. (2014). P-glycoprotein and its Role in Drug-Drug Interactions. *Australian Prescriber*, *37*(4), 137–139. https://doi.org/10.18773/austprescr.2014.050

8) Zhang, B., Cui, W., & Gross, M. L. (2017). Laser-Initiated Radical Trifluoromethylation of Peptides and Proteins: Application to Mass Spectrometry-based Protein Footprinting. *Angewandte Chemie International Edition*, *56*(45), 14007–14010. https://doi.org/10.1002/anie.201706697

9) Inoue, M., Sumii, Y., & Shibata, N. (2020). Contribution of Organofluorine Compounds to Pharmaceuticals. *ACS Omega*, *5*(19), 10633–10640. https://doi.org/10.1021/acsomega.0c00830

10) Song, H. (2017). Research Progress on Trifluoromethyl-Based Radical Reaction Process. *IOP Conference Series: Earth and Environmental Science*, *100*, 012061. https://doi.org/10.1088/1755-1315/100/1/012061

11) Sharp, J. S., Misra, S. K., Persoff, J. J., Egan, R. W., & Weinberger, S. R. (2018). Real Time Normalization of Fast Photochemical Oxidation of Proteins Experiments by Inline Adenine Radical Dosimetry. *Analytical Chemistry*, *90*(21), 12625–12630. https://doi.org/10.1021/acs.analchem.8b02787

12) Cheng, Z., Mobley, C., Misra, S. K., Gadepalli, R. S., Hammond, R. I., Brown, L. S., Rimoldi, J. M., & Sharp, J. S. (2021). Self-organized Amphiphiles are Poor Hydroxyl Radical Scavengers in Fast Photochemical Oxidation of Proteins Experiments. *Journal of the American Society for Mass Spectrometry*, *32*(5), 1155–1161. https://doi.org/10.1021/jasms.0c00457

13) Bern, M., Kil, Y. J., & Becker, C. (2012). Byonic: Advanced Peptide and Protein Identification Software. *Current protocols in bioinformatics*, *Chapter 13*, Unit13.20. https://doi.org/10.1002/0471250953.bi1320s40

14) Fojtík, L., Fiala, J., Pompach, P., Chmelík, J., Matoušek, V., Beier, P., Kukačka, Z., & Novák, P. (2021). Fast fluoroalkylation of proteins uncovers the structure and dynamics of biological macromolecules. *Journal of the American Chemical Society*, *143*(49), 20670–20679. https://doi.org/10.1021/jacs.1c07771

15) Jana, S., Verma, A., Kadu, R., & Kumar, S. (2017). Visible-light-induced oxidant and metal-free dehydrogenative cascade trifluoromethylation and oxidation of 1,6-Enynes with water. *Chemical Science*, *8*(9), 6633–6644. https://doi.org/10.1039/c7sc02556d

16) Koike, T., & Akita, M. (2014). Trifluoromethylation by visible-light-driven photoredox catalysis. *Topics in Catalysis*, *57*(10-13), 967–974. https://doi.org/10.1007/s11244-014-0259-7

17) Li, L., Mu, X., Liu, W., Wang, Y., Mi, Z., & Li, C.-J. (2016). Simple and clean photoinduced aromatic trifluoromethylation reaction. *Journal of the American Chemical Society*, *138*(18), 5809–5812. https://doi.org/10.1021/jacs.6b02782

18) Pan, X., Xia, H., & Wu, J. (2016). Recent advances in photoinduced trifluoromethylation and difluoroalkylation. *Organic Chemistry Frontiers*, *3*(9), 1163–1185. https://doi.org/10.1039/c6qo00153j

19) Xie, B., Sood, A., Woods, R. J., & Sharp, J. S. (2017). Quantitative protein topography measurements by high resolution hydroxyl radical protein footprinting enable accurate molecular model selection. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-04689-3