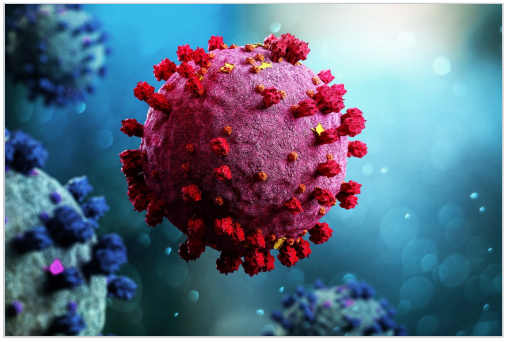
Waters™

Application Note

Comprehending COVID-19: Rapid and Sensitive Characterization of N-Glycans from SARS-CoV-2 Spike Protein

Xiaoxiao Liu, Matthew A. Lauber

Waters Corporation



Need Help? To learn more about how Waters can help you in your efforts against COVID-19, please contact the COVID-19 Innovation Response Team

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

The global COVID-19 pandemic has resulted in extensive efforts to develop vaccines to the novel coronavirus. Identifying vaccine targets relies on robust analytical methods to understand SARS-CoV-2 structural biology. This work is focused on understanding the N-glycosylation profile of the SARS-CoV-2 spike protein, which has emerged as a potential target for vaccine development. As glycans often dictate critical glycoprotein structure and function, understanding SARS-CoV-2 spike protein glycans is essential to further therapeutic development.[1] This work utilizes the GlycoWorks RapiFluor-MS N-Glycan Kit to easily and rapidly detect SARS-CoV-2 spike protein N-Glycans. As a result, 42 major glycan peaks were identified, 2 of which are tentatively assigned as doubly fucosylated. This work motivates further MS/MS analysis to confirm the SARS-CoV-2 spike protein glycosylation profile.

Benefits

Rapid, sensitive, and easy detection of N-glycans

Introduction

During the COVID-19 pandemic, scientists across the globe are working to understand SARS-CoV-2 structural biology. Through this work, the SARS-CoV-2 spike protein has been implicated in viral pathogenesis and has thus emerged as a target for vaccine development. Studies show that neutralizing antibodies interact with the spike protein of the novel coronavirus at both peptide and glycan epitopes.[1,2,3] Understanding spike protein glycans is paramount to appropriate therapeutic development as glycosylation can dictate a significant portion of the structure, function, conformational dynamics, and drug binding site availablility.[1] Therefore, it is critical to characterize glycosylation during the development of new vaccines.

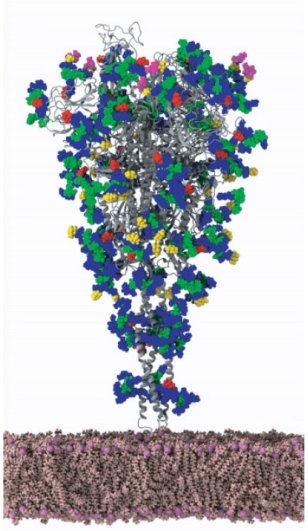


Figure 1. The SARS-CoV-2 spike protein (gray) with glycans modeled on its surface. Lorenzo Casalino, Zied Gaieb, and Rommie Amaro, UC San Diego.

Experimental

N-glycans were released, labeled, and purified for hydrophilic interaction chromatography (HILIC) using the GlycoWorks RapiFluor-MS N-Glycan Kit with optimized DTT reducing conditions for denaturation. HILIC-FLR-MS was performed with an ACQUITY UPLC H-Class LC and a Xevo G2-XS QToF Mass Spectrometer.

LC-MS Conditions

LC system:	ACQUITY UPLC H-Class Bio
Detection:	ACQUITY FLR and Xevo G2-XS QTof
Vials:	QuanRecovery 300 µL
Column(s):	ACQUITY UPLC Glycan BEH Amide, 1.7 µm, 2.1 x 150 mm
Column temp.:	60 ˚C
Sample temp.:	8 ˚C
Injection volume:	1 µL
Flow rate:	0.4 mL/min
Mobile phase A:	50 mM ammonium formate, pH 4.4
Mobile phase B:	Acetonitrile (LC-MS grade)
Gradient:	75-54% Mobile phase B in 35 minutes

For detailed sample preparation information, please see the GlycoWorks Care and Use Manual. For detailed MS conditions, please see Waters Application Note.

GlycoWorks Care and Use Manual	715004903
Waters Application Note	720005850EN

Results and Discussion

42 major glycan peaks were identified (see Figure 2), wherein 2 are tentatively assigned as doubly-fucosylated (see Figure 3). The remaining assignments are: 11 afucosylated glycans, and 29 fucosylated glycans. These glycans can be further grouped into 3 classes, including 6 high mannose glycans, 6 hybrid glycans, 30 complex glycans. These assignments were made based on relative HILIC retention times, glucose unit (GU) values and accurate mass information. Examination by MS/MS analysis and exoglycosidase arrays is warranted in order to confirm identifications.

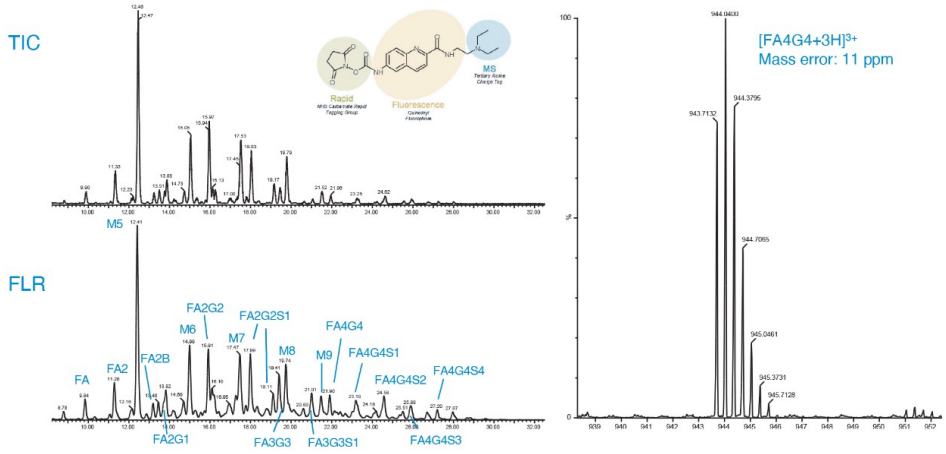


Figure 2. Identification of 42 Major Glycan Peaks by MS.

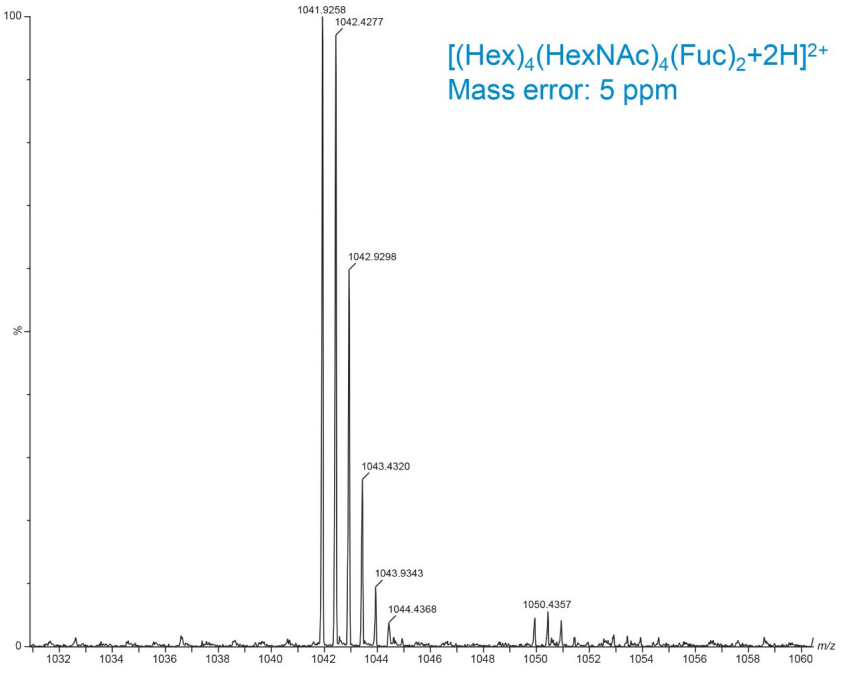


Figure 3. MS Spectra of Doubly-Fucosylated Glycans.

Conclusion

Because the SARS-CoV-2 spike protein is implicated in viral pathogenesis, it has become a target for vaccine development. Efficient therapeutic development relies on a solid structural and functional understanding of the SARS-CoV-2 spike protein target. Understanding the glycan profile is critical to a complete structural and functional understanding of SARS-CoV-2 spike protein. As a result, rapid and accurate glycan analysis is necessary to identify and develop promising new COVID-19 therapies. This work demonstrates the ability to rapidly and easily detect SARS-CoV-2 N-glycans. 42 major glycan peaks were identified. Interestingly, 2 peaks were assigned as doubly-fucosylated. This curious finding calls for further corroboration through examination by MS/MS and exoglycosidase arrays.

References

1. Novokmet, Mislav *et al.* Understanding glycans in COVID-19 drug design. https://www.genengnews.com/insights/understanding-glycans-in-covid-19-drug-design/

2. Pinto, D. *et al.* Structural and functional analysis of a potent sarbecovirus neutralizing antibody. *bioRxiv* 2020.04.07.023903 (2020). doi: https://doi.org/10.1101/2020.04.07.023903

3. Stawiski, E.W. *et al.* Human ACE2 receptor polymorphisms predict SARS-CoV-2 susceptibility. *bioRxiv* 2020.04.07.024752 (2020). doi: https://doi.org/10.1101/2020.04.07.024752

720006914, May 2020