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Application Note

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

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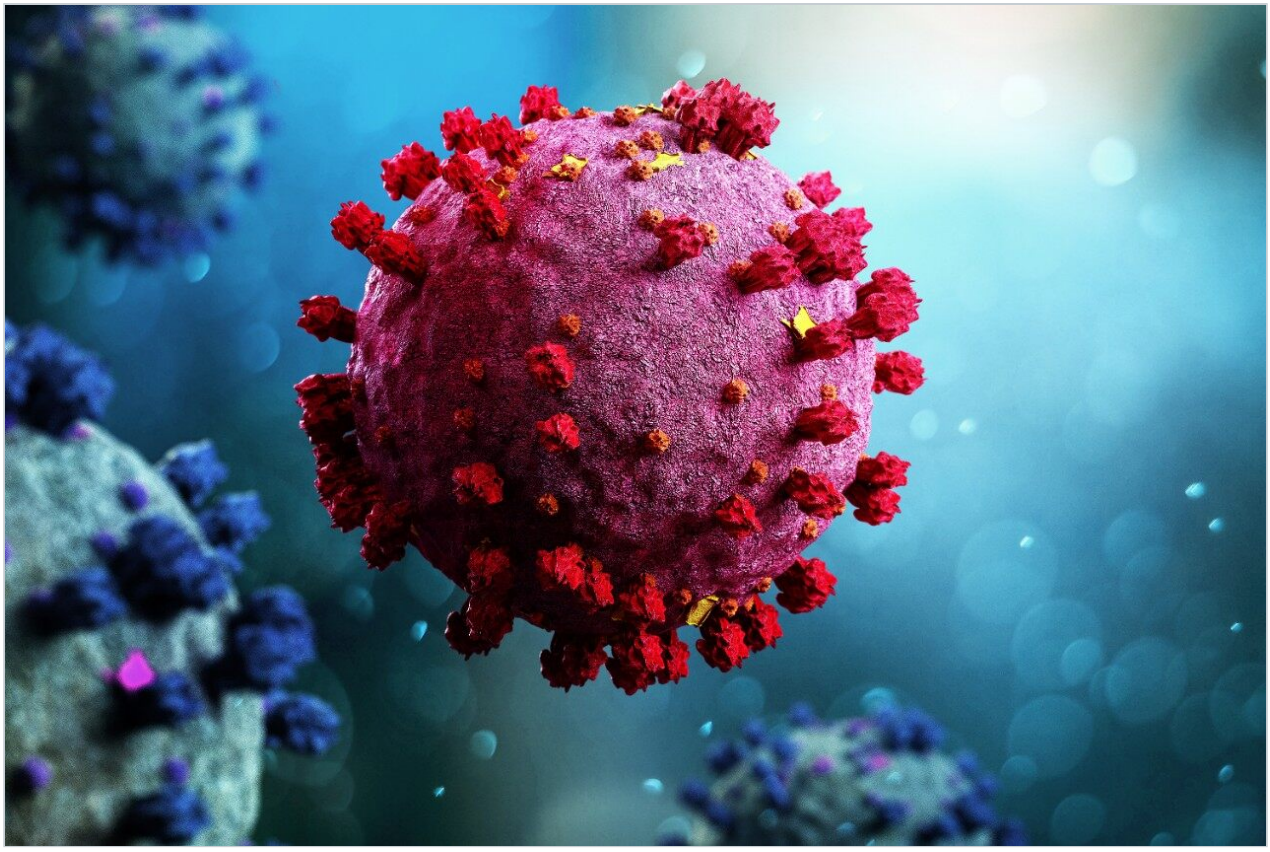

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This is an Application Brief and does not contain a detailed Experimental section.

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 *Rapi*Fluor-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, two 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.

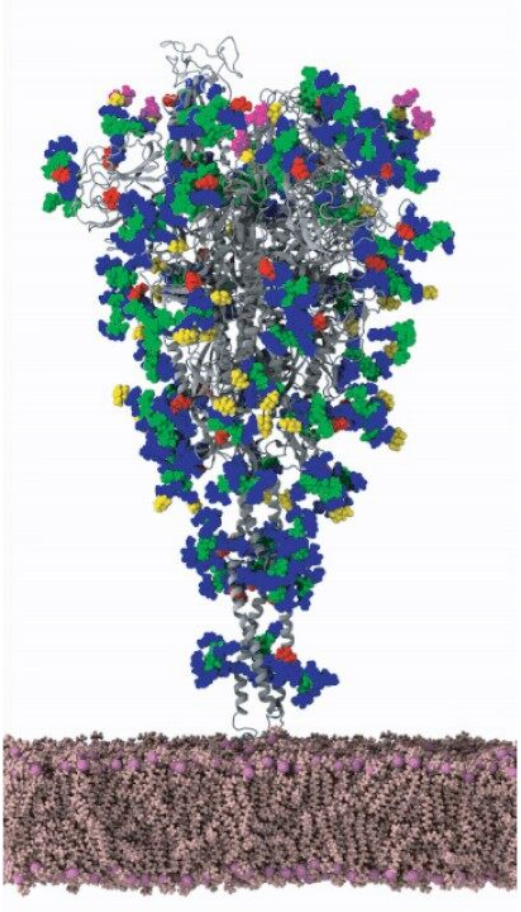

Figure 1. The SARS-CoV-2 spike protein (gray) with glycans modeled on its surface. Lorenzo Casalino, Zied Gaieb, and Rommie Amaro, UC San Diego.

Experimental

N-glycans were released, labeled, and purified for hydrophilic interaction chromatography (HILIC) using the GlycoWorks *Rapi*Fluor-MS N-Glycan Kit with optimized DTT reducing conditions for denaturation. HILIC-FLR-MS was performed with an ACQUITY UPLC H-Class Bio System 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 < https://www.waters.com/webassets/cms/support/docs/715004903. >

Waters Application Note	720005850EN < https://www.waters.com/webassets/cms/library/docs/720005850e >

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.

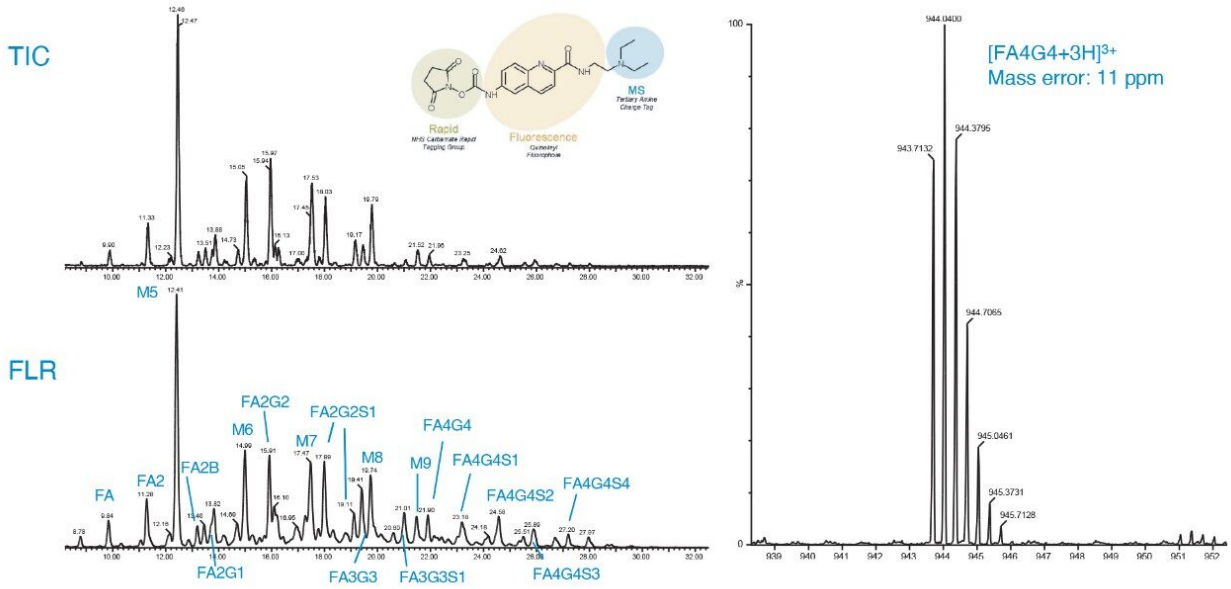

Figure 2. Identification of 42 major glycan peaks by MS.

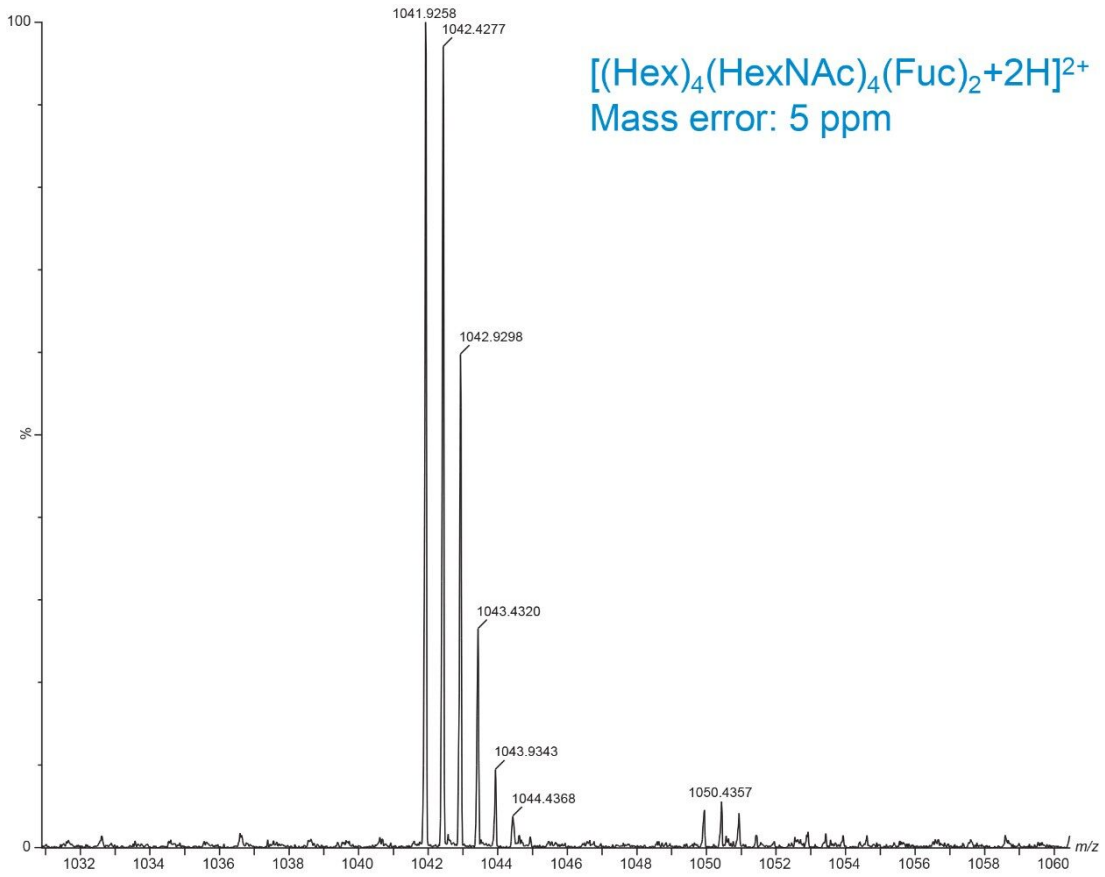

Figure 3. MS spectra of doubly-fucosylated glycans.

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, two 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/ < 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

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720006914, Revised November 2020