

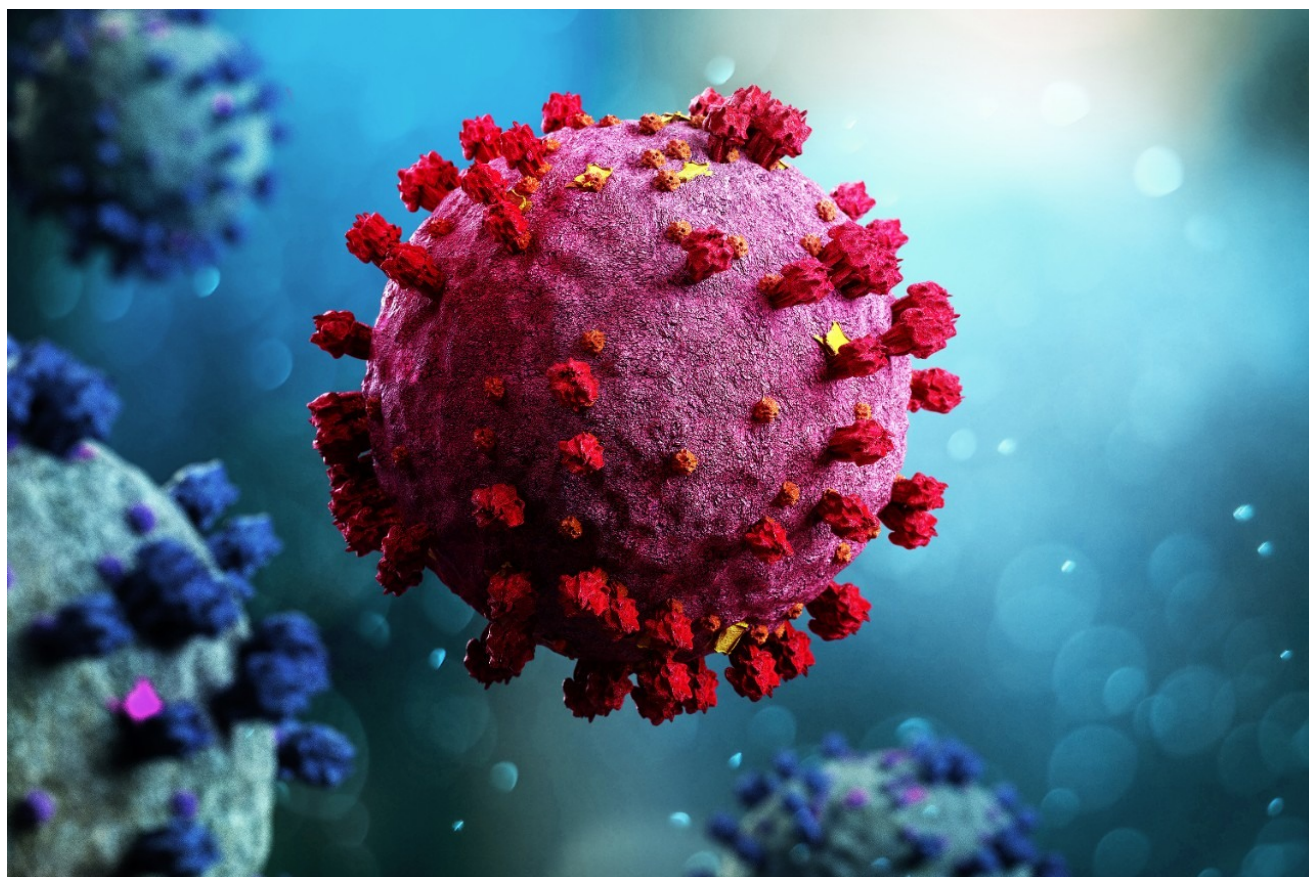
# Waters™

應用手冊

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

Xiaoxiao Liu, Matthew A. Lauber

Waters Corporation



需要帮助?

如需详细了解沃特世如何为您抗击新型冠状病毒肺炎(COVID-19)提供助力, 请联系

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.<sup>1</sup> 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.<sup>1,2,3</sup> 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 availability.<sup>1</sup> Therefore, it is critical to characterize glycosylation during the development of new vaccines.

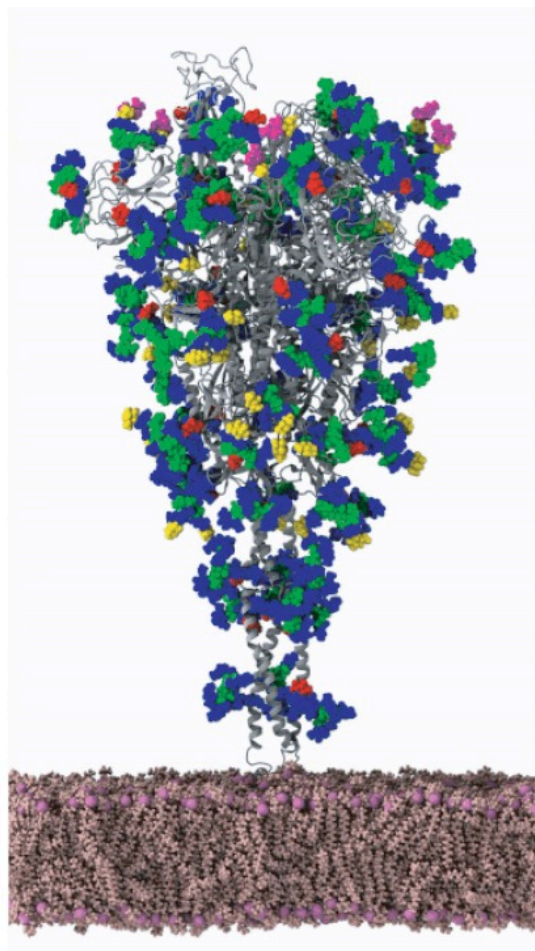


Figure 1. The SARS-CoV-2 spike protein (gray) with glycans modeled on its surface. Lorenzo Casalino, Zied Gaieb, and Rommie Amaro, UC San Diego.

## 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	<a href="https://www.waters.com/webassets/cms/support/docs/715004903.pdf">715004903 &lt;</a> <a href="https://www.waters.com/webassets/cms/support/docs/715004903.pdf">https://www.waters.com/webassets/cms/support/docs/715004903.pdf</a> <a href="https://www.waters.com/webassets/cms/support/docs/715004903.pdf">≥</a>
Waters Application Note	<a href="https://www.waters.com/webassets/cms/library/docs/720005850en.pdf">720005850EN &lt;</a> <a href="https://www.waters.com/webassets/cms/library/docs/720005850en.pdf">https://www.waters.com/webassets/cms/library/docs/720005850en.pdf</a> <a href="https://www.waters.com/webassets/cms/library/docs/720005850en.pdf">≥</a>

## 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.

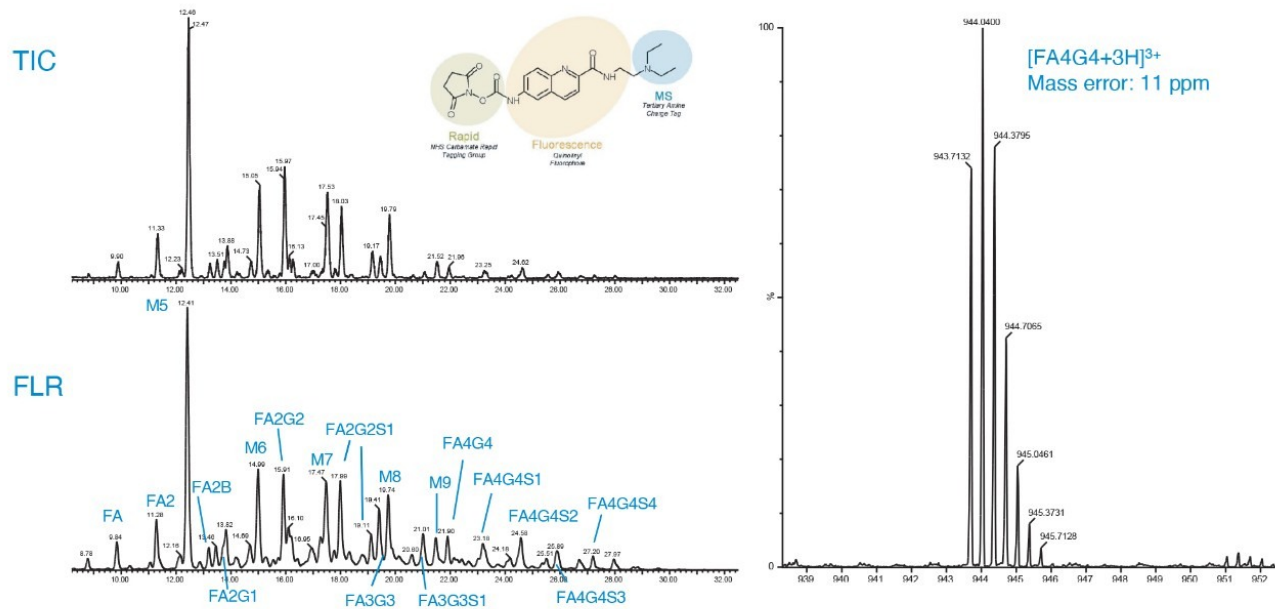


Figure 2. Identification of 42 major glycan peaks by MS.

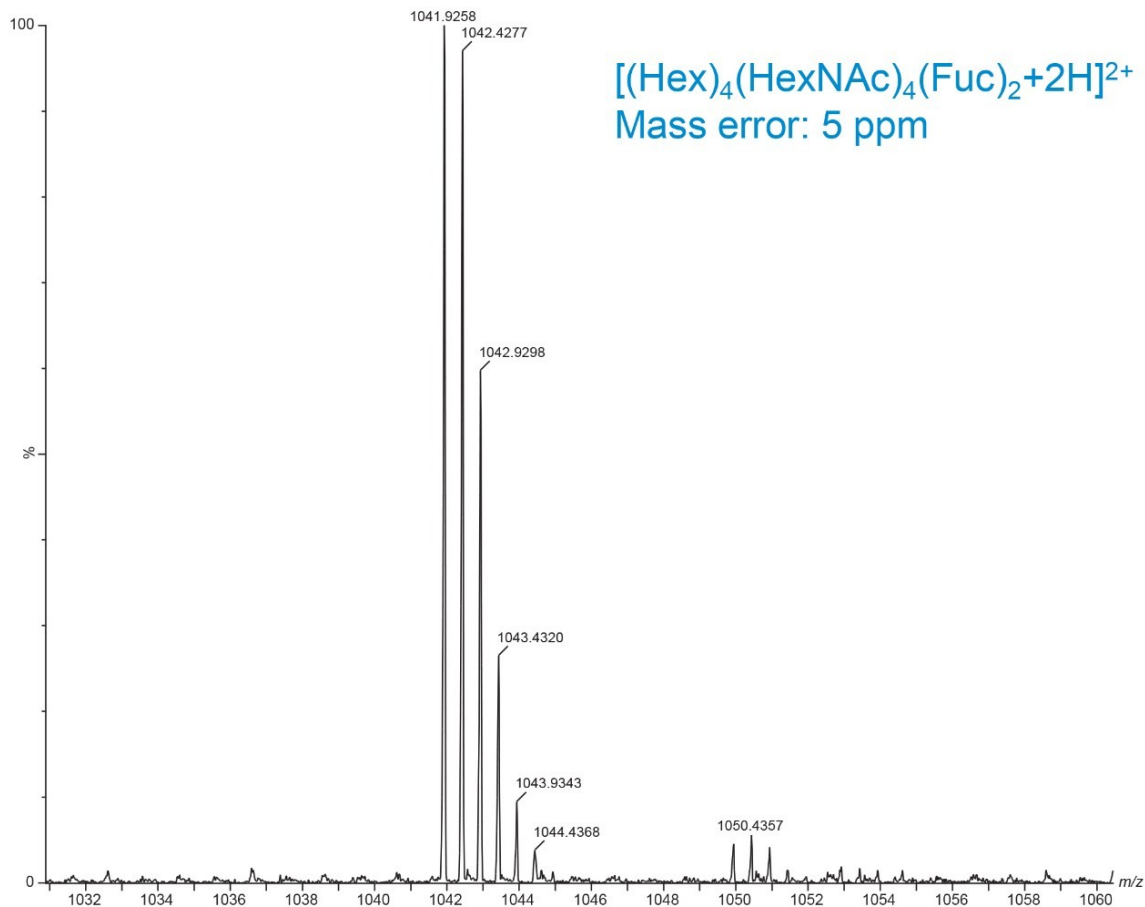


Figure 3. MS spectra of doubly-fucosylated glycans.

## 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>

## Featured Products

- [ACQUITY UPLC H-Class PLUS Bio System <https://www.waters.com/10166246>](https://www.waters.com/10166246)
- [Xevo G2-XS QToF Quadrupole Time-of-Flight Mass Spectrometry <https://www.waters.com/134798222>](https://www.waters.com/134798222)
- [ACQUITY UPLC FLR Detector <https://www.waters.com/514222>](https://www.waters.com/514222)

720006914, Revised November 2020



©2020 Waters Corporation. All Rights Reserved.