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Application Note

Comprehending COVID-19: Preliminary Examination of the SARS-CoV-2 Spike Protein by Peptide Mapping

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

Abstract

The SARS-CoV-2 spike protein has rightly garnered attention as a leading candidate for protein-based vaccines for COVID-19. Multiple studies have shown that neutralizing antibodies, from convalescent coronavirus patients, bind to the spike protein at both peptide and peptidoglycan epitopes. Peptide mapping analyses by liquid chromatography and mass spectrometry might therefore prove to be very useful during the development and QC analysis of new protein-based vaccine candidates for the novel coronavirus.

Benefits

- Robust and accurate confirmation of primary sequence and monitoring of post-translational modifications as performed with the BioAccord LC-MS System
- Easy-to-use and streamlined data analysis with UNIFI Scientific Information System

Introduction

SARS-CoV-2, like other coronaviruses, relies on a spike protein to initiate host cell entry.[1] This novel coronavirus spike protein is a ~540 kDa homotrimeric glycoprotein that is heterogeneously decorated with 66 N-glycan sites, which has recently been characterized by Watanabe et al. O-glycosylation on the protein, while predicted by computational modeling, has not yet been as thoroughly detailed.[2,3] Despite its complexity, it is worthwhile to subject the spike protein to multiple analytical tests to offer new insights into SARS-CoV-2 virology. These new insights may lead to more robust development and manufacture of new vaccine candidates for COVID-19. Comprehensive characterization and monitoring of recombinant proteins by peptide mapping are techniques frequently used for the identification and tracking of post-translational modifications, like the previously mentioned glycans. Here, we have applied a tryptic peptide mapping approach to analyze a recombinant SARS-CoV-2 spike protein using a BioAccord LC-MS System to confirm sequence information and to make a preliminary assessment of site-specific glycan heterogeneity.

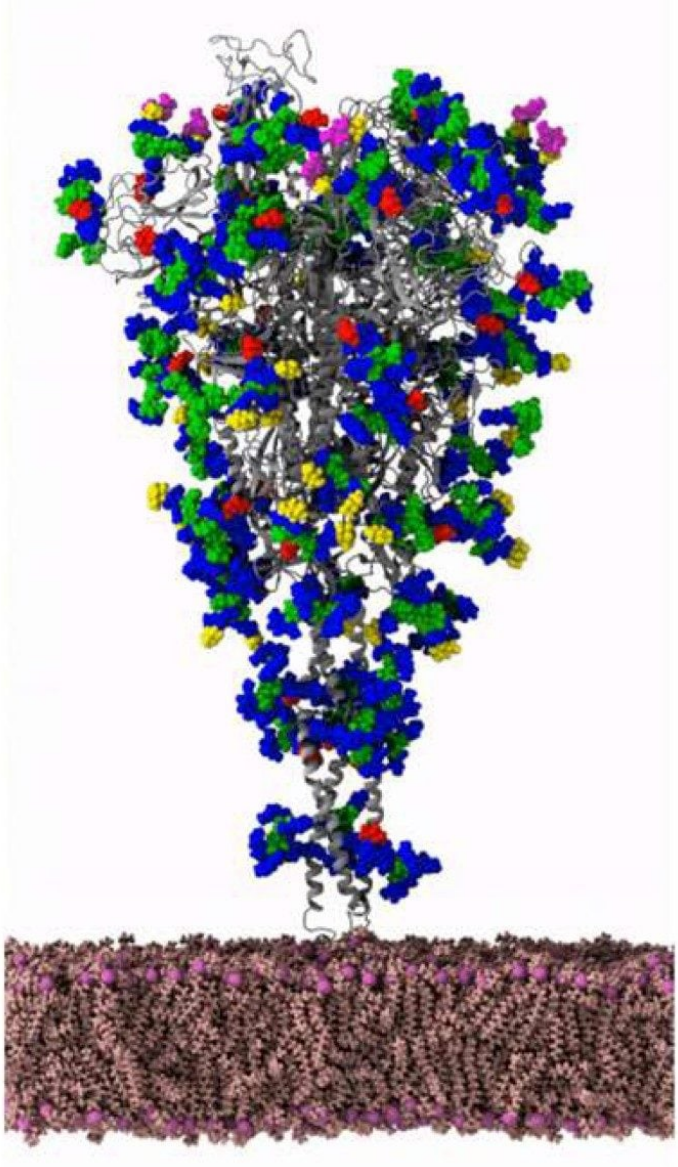

Figure 1. The SARS-CoV-2 spike protein (gray) with glycans modeled on its surface. Lorenzo Casalino, Zied Gaieb, and Rommie Amaro, UC San Diego.

Experimental

Recombinant SARS-CoV-2 spike protein, expressed and purified from HEK293 cells, was denatured with 8 M GuHCl, reduced and alkylated and then desalted with sizing media prior to being digested with trypsin overnight. To one aliquoted sample, PNGase F was added to hydrolyze away N-glycans. The resulting peptides were analyzed through the integrated peptide mapping workflow within the BioAccord LC-MS System. Data were processed with UNIFI to assign peptide identifications, confirm amino acid sequences with high energy MS fragmentation data, and to identify post-translational modifications.

LC-MS Conditions

LC-MS system:	BioAccord LC-MS
Detection:	ACQUITY TUV
Vials:	QuanRecovery, 96-well
Column(s):	ACQUITY UPLC Peptide BEH C_{18} Column (p/n 186003555)
Column temp.:	65 °C
Sample temp.:	6 °C
Injection volume:	10 µL
Flow rate:	0.25 mL/min
Mobile phase A:	0.1% formic acid in H_2O
Mobile phase B:	0.1% formic acid in acetonitrile
Ionization mode:	ESI+
Acquisition range:	*m/z* 50–2000

Capillary voltage:	1.2 kV
Collision energy:	60–130 V
Cone voltage:	30 V

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
0	0.25	99	1	6
5	0.25	99	1	6
65	0.25	60	40	6
68	0.25	30	70	6
70	0.25	30	70	6
71	0.25	99	1	6
85	0.25	99	1	6

Data Management

Informatics:	UNIFI Scientific Information System v1.9.4

Results and Discussion

With the conditions described in peptide mapping experiments, approximately 90% sequence coverage was achieved for the recombinant SARS-COV-2 spike protein (Figure 2). The matching criteria for peptide identification in UNIFI was set with 15 ppm mass accuracy for a precursor and for greater than or equal to 3 fragment b or y ions to be found. For glycopeptides, criteria were relaxed to greater or equal to 1 fragment given that the predicted lability of glycosidic bonds results in the scarcity of peptide backbone cleavages. Ultimately, more than 90% of the previously reported N- and O-glycosites were successfully assigned. Signature N-glycopeptides were identified with high confidence, especially for the abundant oligomannose type N234 glycopeptide, which is implicated in the shielding of receptor binding sites (Figure 3). O-glycosylation at T323 was also confirmed in corroboration with a recent report.[4] These data show the potential of BioAccord based peptide mapping workflow to map out the glycosylation sites/glycoforms for a complex recombinant glycoprotein. Future work with additional (different) enzyme treatment is likely to expand the coverage of information obtained from trypsin-based preparation.

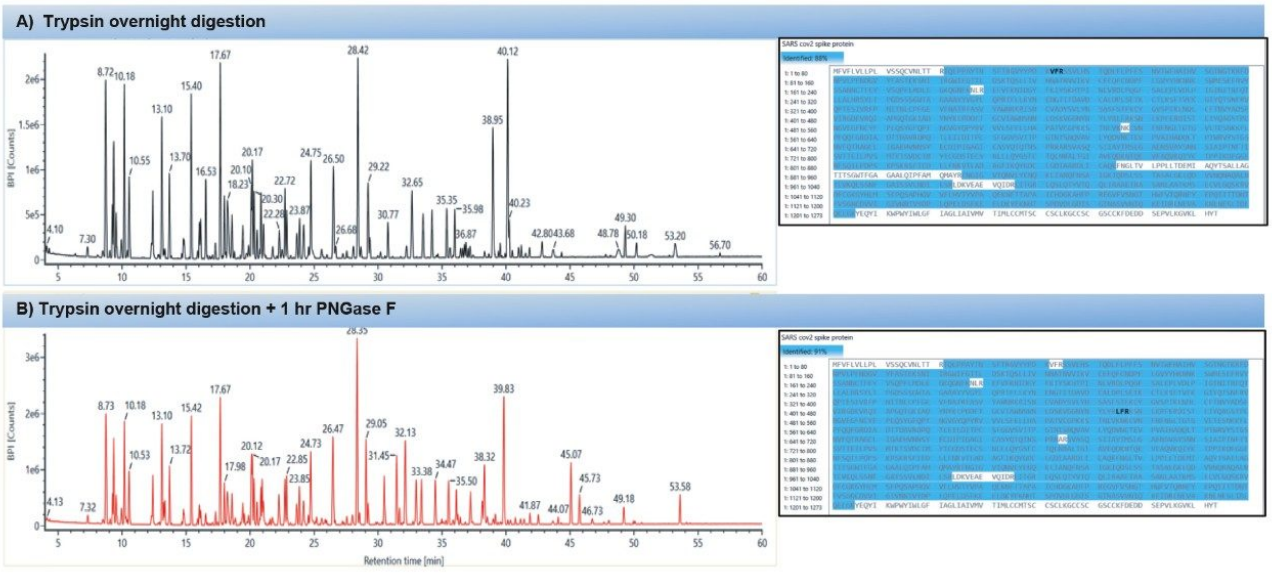

Figure 2. Peptide maps of the SARS-COV-2 spike protein generated after A) trypsin digestion; B) trypsin digesion + 1 hr PNGase F. Inserts show sequence coverages as processed by UNIFI.

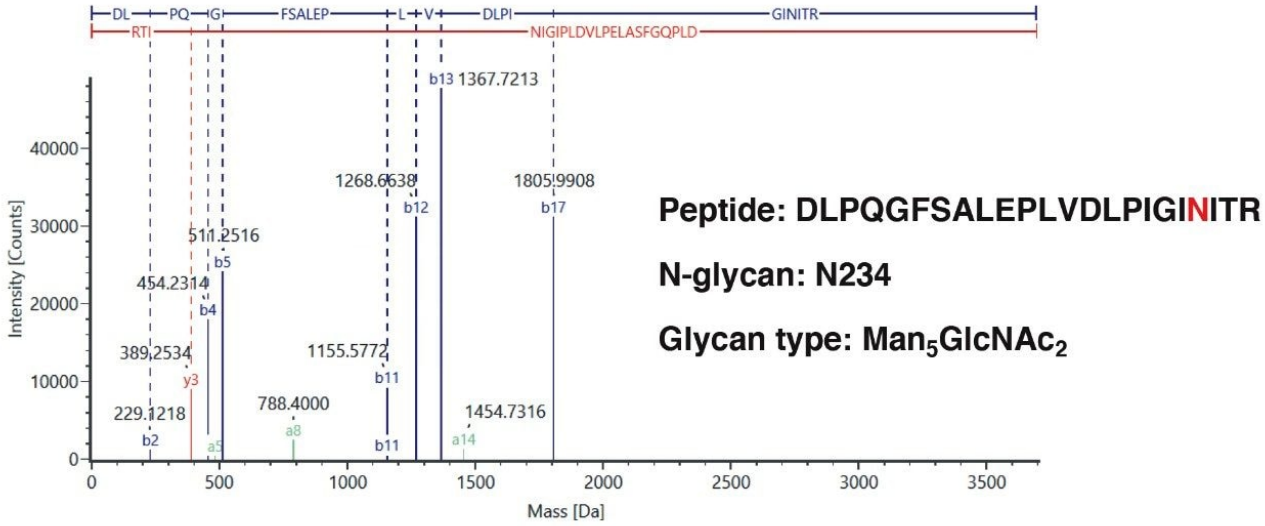

Figure 3. The assignment of one N-glycopeptide at site N234 based on the high-energy fragmentation data.

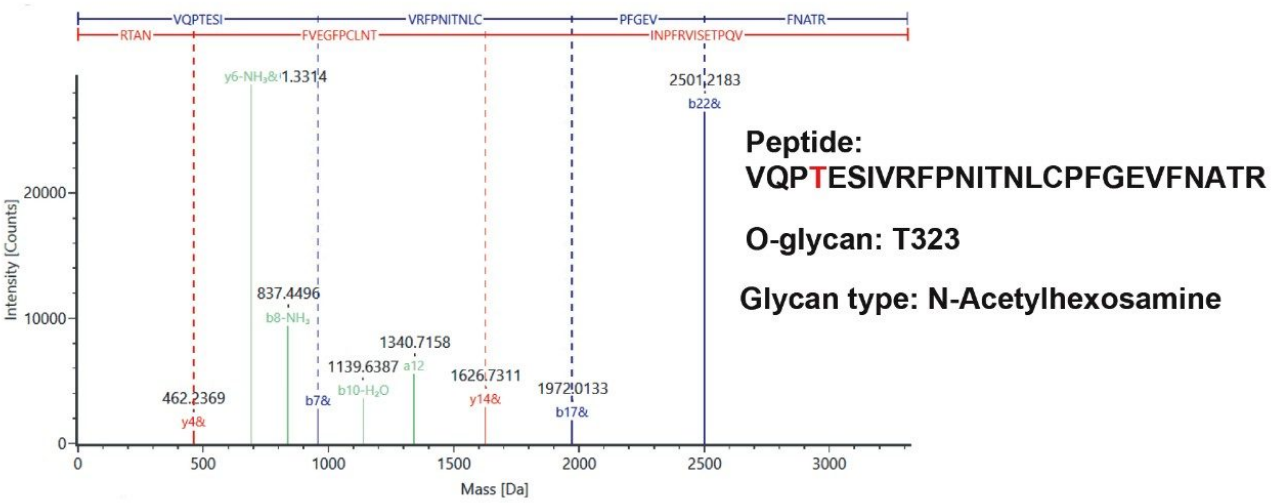

Figure 4. The assignment of one O-glycopeptide at site T323 based on the high-energy fragmentation data.

Conclusion

Efficacious development of COVID-19 vaccines requires thorough analytical characterization of protein antigens. To induce desired levels of immunological response, it is critical for protein-based COVID-19 vaccine candidates to reflect the natural antigen structural features. To meet this analytical challenge associated with the novel coronavirus, we have applied an easy-to-use and streamlined peptide mapping workflow on the BioAccord System. Using the BioAccord, we have accurately and reproducibly confirmed sequence coverage and have started to profile PTMs of SARS-CoV-2 spike protein. The capabilities of this approach will allow glycosylation, oxidation, deamidation, and clipping events to be monitored throughout process development and manufacturing stages.

References

1. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*. 2020;181(2):281–292.e6.
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4. Shajahan A, Supekar NT, Gleinich AS, Azadi P. Deducing the N- and O-glycosylation Profile of the Spike Protein of Novel Coronavirus SARS-CoV-2 [published online ahead of print, 2020 May 4]. *Glycobiology*. 2020;cwaa042.

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