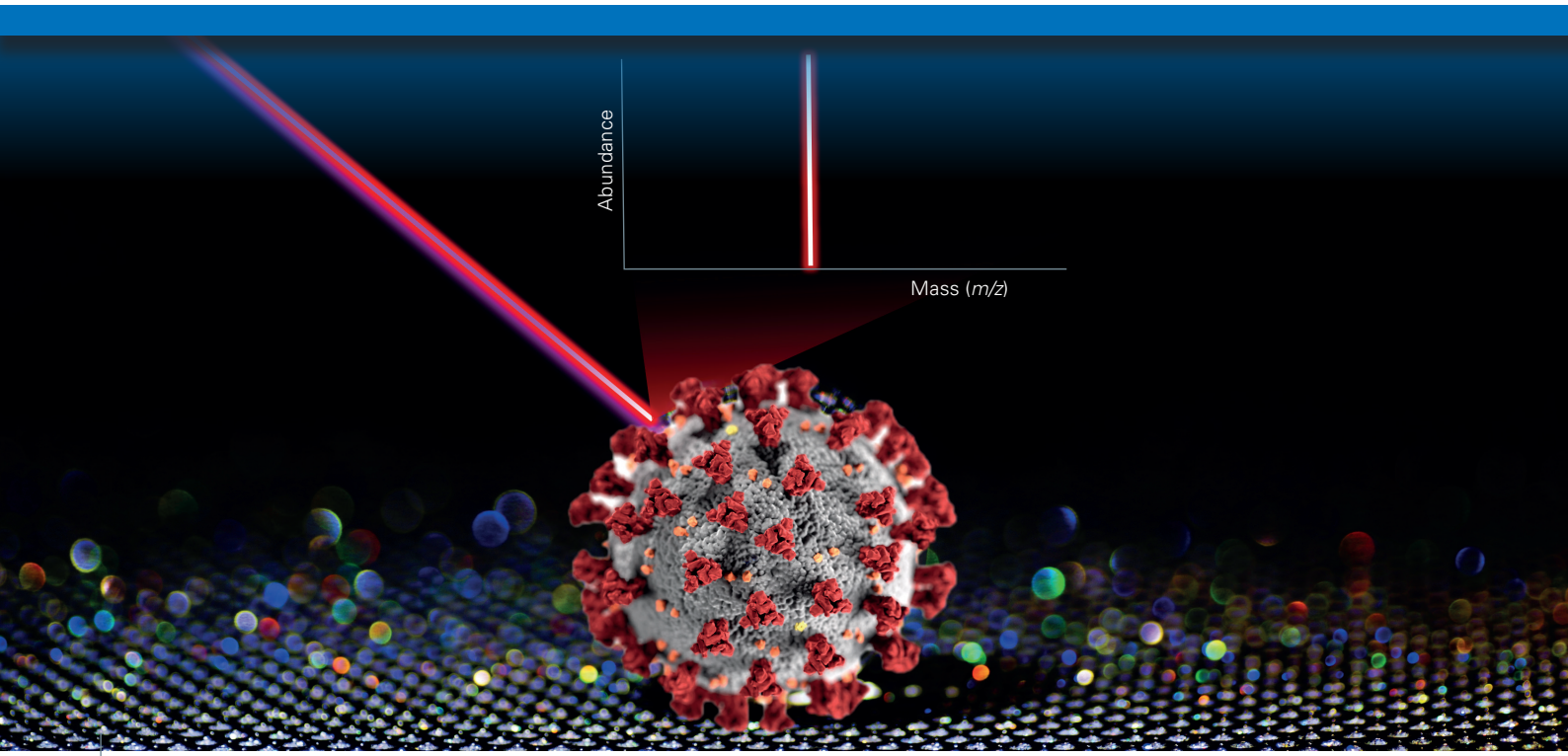

Initial Development of SARS-CoV-2 Antigen Assay Using BAMS Technology and MALDI-TOF

This work is focused on the development of a new SARS-CoV-2 spike glycoprotein assay based on Bead Assisted Mass Spectrometry (BAMS) assay platform with detection by MALDI-TOF MS.

Abstract

The covalent attachment of Affimers with protein specificity to BAMS magnetic beads in order to affinity capture SARS-CoV-2 spike glycoprotein subunit 1 (S1 glycoprotein) is a novel concept creating a unique foundation for this assay development. The selectively captured S1 glycoprotein was eluted and analyzed as the intact protein and digested on-bead for bottom-up workflow using a high performance rapifleX TOF/TOF system. Afterwards the novel assay was adapted for measurements on a microflex instrument platform.

Introduction

Since the start of the COVID-19 global pandemic incredible amount of time and effort has been dedicated to the development of assays and diagnostic tests for SARS-CoV-2. Currently most commercially available molecular diagnostic tests are based on real-time reverse

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SARS-CoV-2, coronavirus, antigen assay, Affimer, BAMS, MALDI-TOF

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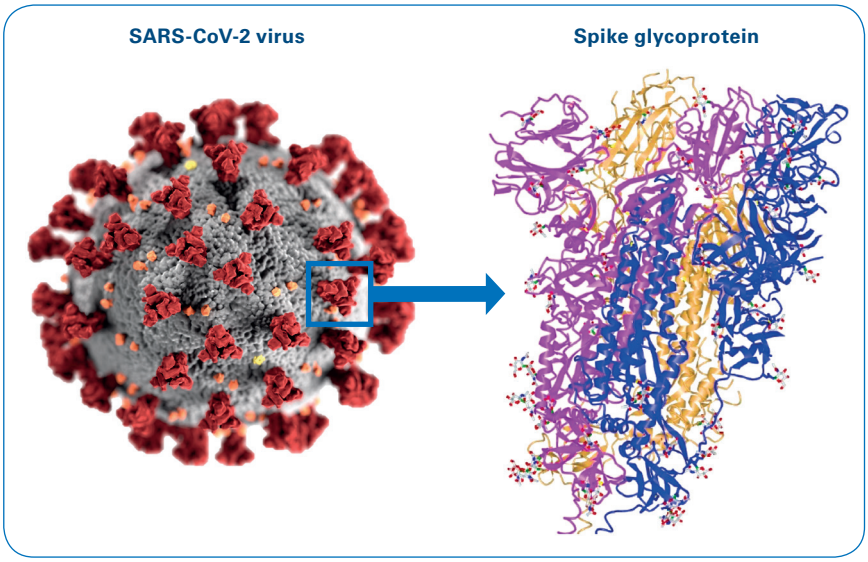

Figure 1: SARS-CoV-2 spike glycoprotein is shown localized on the surface of the virus particle in red (left) and as the ribbon structure of the trimeric protein (right).

transcription-polymerase chain reaction (RT-PCR) technology targeting various SARS-CoV-2 genomic regions [1]. Relatively few antigen tests have been approved so far. Most of them are based on lateral flow immunoassays [1]. Here we describe the development of a novel SARS-CoV-2 antigen assay using a BAMS assay platform with detection by MALDI-TOF. The BAMS assay platform is an approach for developing targeted proteomic assays of almost any protein target for which a specific affinity reagent (i.e., antibody or Affimer) is available. This approach utilizes multiplex immuno-affinity capture [2, 3]. Outstandingly, this assay uses an Affimer with S1 glycoprotein specificity instead of a monoclonal antibody for affinity capture.

Experimental

BAMS assay for SARS-CoV-2 S1 glycoprotein

Recombinant SARS-CoV-2 S1 glycoprotein was obtained from Acro Biosystems (Newark, DE). BAMS high binding capacity magnetic beads were functionalized by covalently linking Affimer (Avacta Life Sciences, Wetherby, UK) specific for S1 glycoprotein (SKU# R0502, Adeptrix, Beverly, MA) [4]. Affinity capture of S1 glycoprotein was accomplished by incubating in PBS buffer containing digested BSA followed by a washing step. Bead-bound Affimer with no added S1 glycoprotein was used as the negative control. Intact S1 glycoprotein was eluted with 2,5-dihydroxyacetophenone (DHAP) matrix solution onto disposable steel MALDI plates that matched the dimensions of BAMS microarray slides or onto an MSP 96 ground steel MALDI plate.

S1 glycoprotein was digested on-bead with trypsin for one hour at 37˚C followed by elution with alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix solution onto the same MALDI plates as described above.

MALDI-TOF acquisition and processing

Initial assay development was done using a Bruker rapifleX TOF/TOF equipped with a 10 kHz smartbeam 3D laser. Eluted intact S1 glycoprotein was analyzed in linear mode using the instrument method with the mass range expanded to 10-300 kDa. On-bead digest of S1 glycoprotein was analyzed on a rapifleX in reflector mode using the standard instrument method with the mass range expanded to 700-7000 Da followed by TOF/TOF measurements using the standard MS/MS method.

Subsequent measurements were performed on a Bruker microflex smart LS equipped with a smartbeam laser operating at 200 Hz and on a Bruker microflex LT equipped with a 60 Hz nitrogen laser. All microflex measurements were carried out in linear mode. Intact S1 glycoprotein was measured on a microflex smart LS using the standard instrument method with the same mass range as in the rapifleX measurements. On-bead digests of S1 glycoprotein were measured on a microflex smart LS and microflex LT using the standard instrument methods for peptide mixtures with the mass range set to 700-7000 Da.

Processing was done using flexAnalysis software. S1 glycoprotein digest spectra were post-processed in BioPharma Compass and BioTools software packages (Bruker Scientific).

Results

SARS-CoV-2 spike glycoprotein (S) is one of the most important targets in the development of antigen tests and potential antiviral therapeutics. Recombinant S1 glycoprotein was chosen as the antigen in this test development project (Figure 1). The recombinant S1 glycoprotein contains 688 amino acid residues including the sequence [16-685] from S glycoprotein plus 18 residue polyhistidine tag containing 10 consecutive His residues at the C-terminus. The initial assay development was done on a rapifleX MALDI-TOF/TOF system with subsequent development measurements accomplished on microflex systems that will facilitate the future development stages in clinical environment.

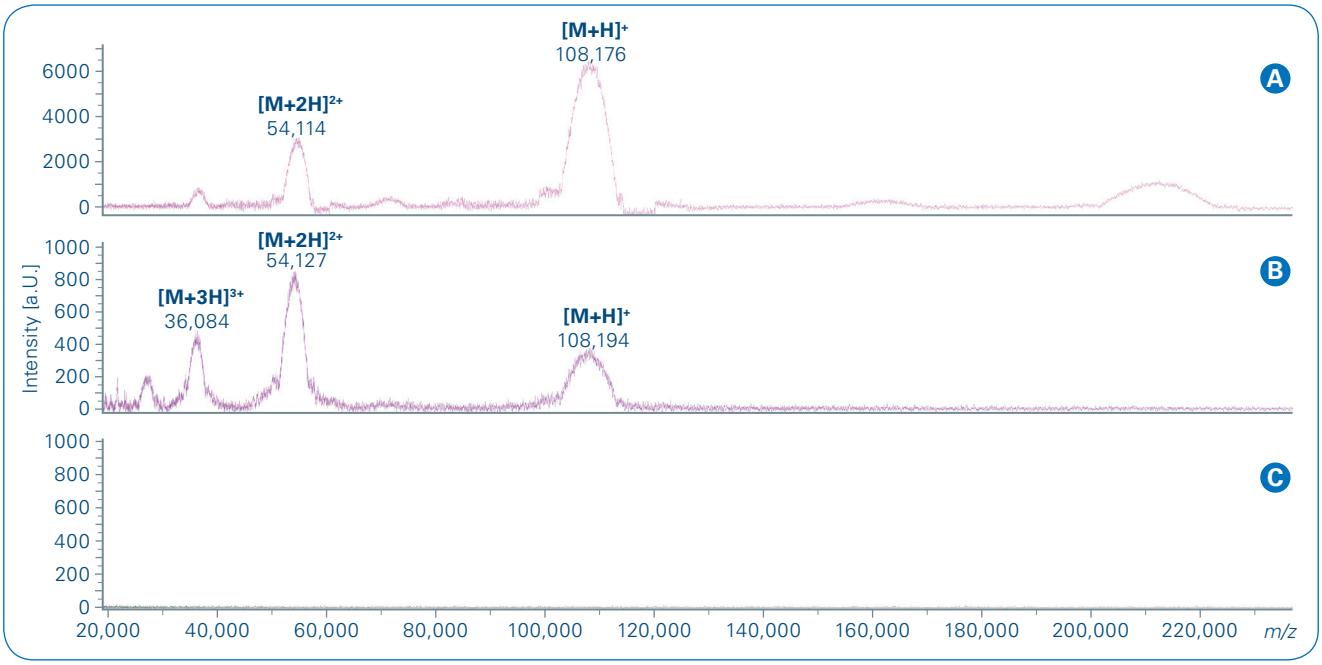

Figure 2: MALDI-TOF spectra of intact S1 glycoprotein eluted from 5 BAMS beads and acquired on a rapifleX (A), eluted from 1 BAMS bead and acquired on a microflex smart LS (B) and the negative control acquired on a microflex smart LS (C).

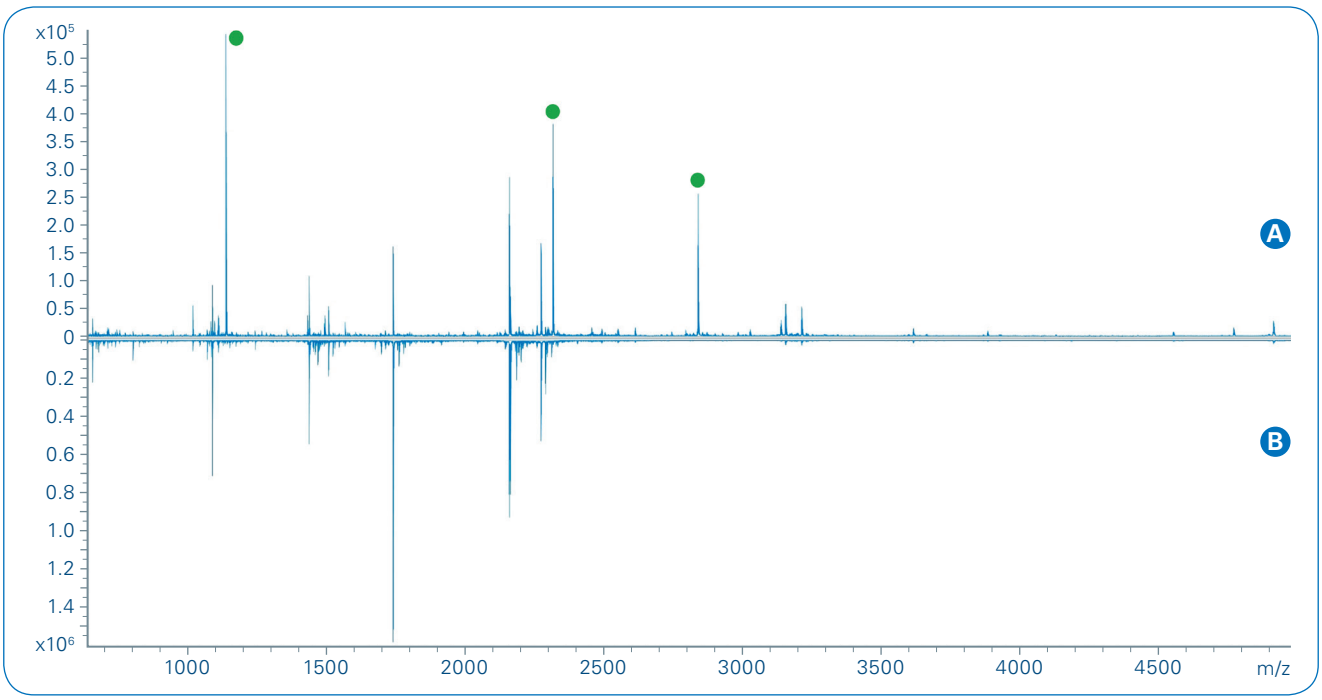

Figure 3: Butterfly plot of S1 glycoprotein and Affimer on-bead digest with the top 3 S1 glycoprotein peptides highlighted with green circles (A) and Affimer control on-bead digest (B) spectra acquired in reflector mode on a rapifleX.

The first step in the analytical strategy of the assay development was to detect intact S1 glycoprotein eluted from specially functionalized BAMS beads. The best results were achieved by using the sample preparation with DHAP as a matrix. S1 glycoprotein produces a very broad signal for $[M+H]^+$ ion due to the presence of multiple glycoforms (Figure 2).

The next step in the development was to detect the tryptic peptides from the on-bead digest. Detection on the peptide level is expected to improve the limit of detection of the assay. The S1 glycoprotein affinity

Table 1: Sequence coverage of eluted S1 glycoprotein digest measured on a microflex smart LS and a microflex LT

S1 glycoprotein BAMS assay	microflex smart LS Sequence coverage MS (%)	microflex LT Sequence coverage MS (%)
Elution from 1 bead	28.5	28.2
Elution from 5 beads	39.4	39.1

bound to the Affimer and the Affimer covalently attached to BAMS beads undergo digestion. The Affimer only digest spectrum (negative control) acquired in reflector mode was used as the reference in the butterfly plot of the two spectra (Figure 3). There are clear differences between the two spectra across the whole mass range from 800 Da to 5000 Da.

The annotated digest spectrum of S1 glycoprotein acquired using eluate from 5 beads is shown in Figure 4. The MS sequence coverage was 65.0% and the MS/MS sequence coverage was 22.5% (Figure 5). Representative MALDI-TOF/TOF spectra of two peptides are shown in Figure 6. Both spectra have excellent sequence coverage with the complete series of the C-terminal y-type fragment ions and a substantial part of the series of the N-terminal b-type fragment ions.

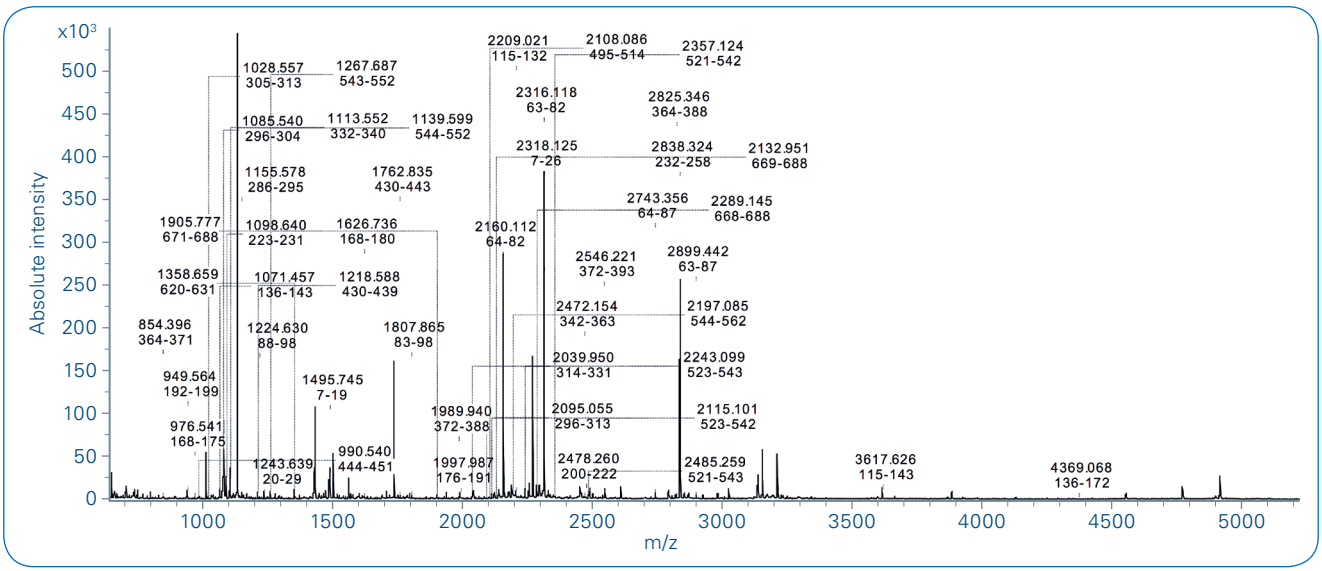

Figure 4: The spectrum of on-bead digest annotated with S1 glycoprotein sequence confirmed selective capture and elution from BAMS beads. It was acquired on a rapifleX in reflector mode.

Sequence coverage MS 65.0%
Sequence coverage MS/MS 22.5%

Figure 5: The MS and MS/MS sequence coverage from on-bead digestion of S1 glycoprotein.

The last part at this early stage in the development was evaluating feasibility of using the microflex platform for detection. The results for intact S1 glycoprotein eluted from 1 bead obtained on a microflex smart LS are comparable to the results that were obtained on a rapifleX (Figure 2B). The sequence coverages on the digest level obtained in linear mode on a microflex smart LS and microflex LT were predictably lower than those obtained on the rapifleX but still allow the possibility of the assay transfer to the microflex platform. Each of the sequence coverage values was averaged from two measurements (Table 1).

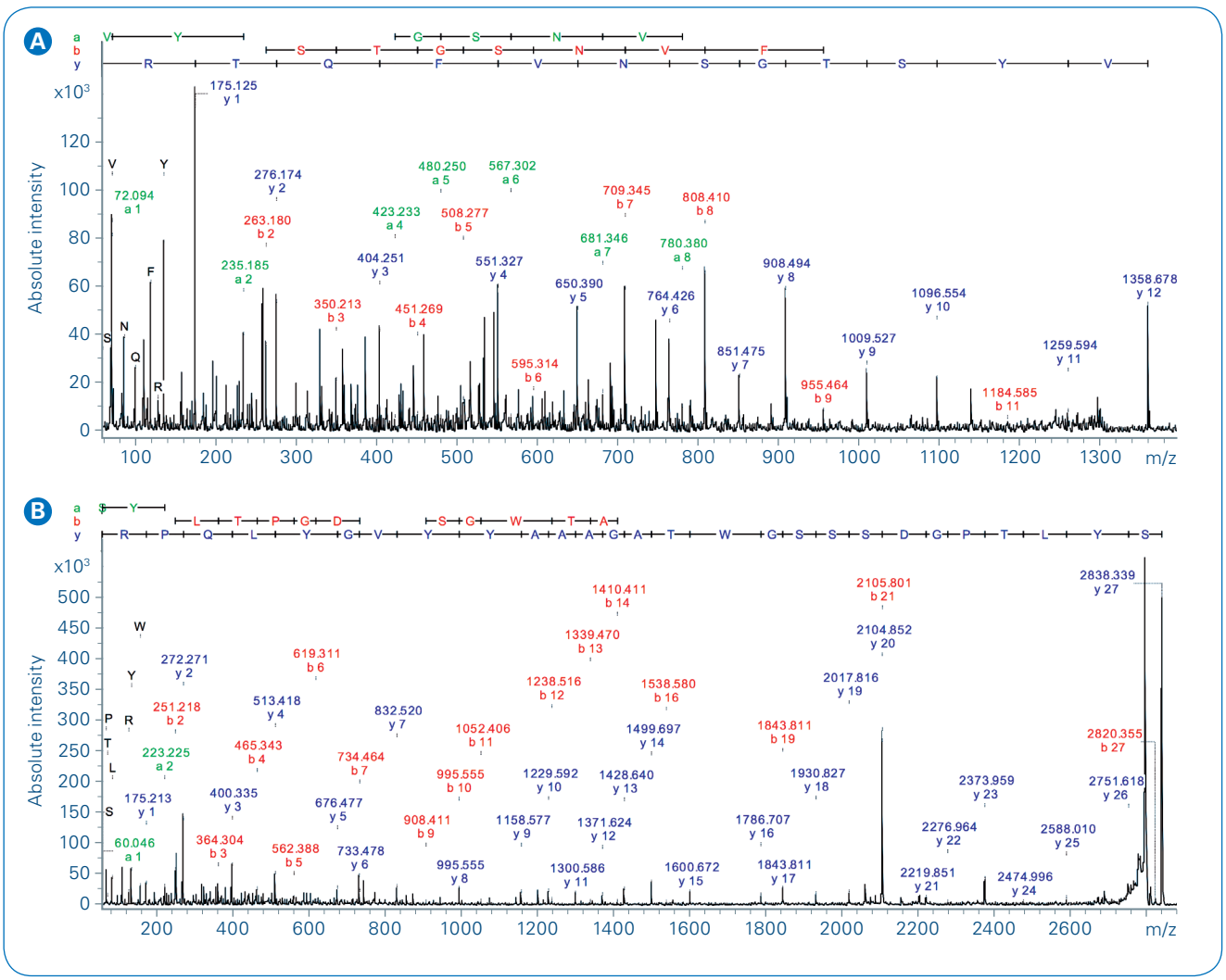

Figure 6: On-bead digestion of S1 glycoprotein. Representative MALDI-TOF/TOF spectra of peptides at m/z *1358.670* (A) *and at* m/z *2838.337* (B).

Conclusion

- Novel SARS-CoV-2 spike glycoprotein assay was developed using recombinant S1 glycoprotein affinity capture on BAMS magnetic beads uniquely functionalized with a high specificity Affimer.
- Eluted S1 glycoprotein was successfully detected as the intact protein and as the trypsin digest using on-bead digestion in the bottom-up approach.
- The assay for S1 glycoprotein was transferred from the high performance rapifleX TOF/TOF instrument platform to the microflex platform that will facilitate future testing of the assay in clinical settings.

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