UTILISATION OF CYCLIC ION MOBILITY WITH MULTIPLE PASS ACQUISITION FOR THE ANALYSIS OF GLYCOPEPTIDES AND GLYCOFORMS ASSOCIATED WITH SARS-COV-2

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to be responsible for the large-scale epidemic globally. The SARS-CoV-2 S protein is highly conserved and involved in multiple processes, including receptor recognition and viral attachment. The viral S protein is modified by glycosylation which may be implicated in immune evasion from the host immune system by shielding the protein surface from detection by antibodies, affecting the ability of the host to mount an effective adaptive immune response. It has therefore become an important target for vaccine research. Here, we demonstrate the utility of Cyclic™ IMS (cIMS) for in-depth glycopeptide characterisation using the multi-pass feature to separate co-eluting glycoforms related to the SARS-CoV-2 S1 protein.

CONCLUSION

- **Utilising an ion mobility workflow, consisting of high mobility resolution allows for high protein sequence coverage and the identification of multiple glycoforms.**
- **Single pass HDMSE data acquired for the SARS-COV-2 spike protein revealed a number of glycoforms (predominantly focusing on N-linked glycosylation)**
- **Multi-pass functionality of the Cyclic™ IMS enabled differentiation of glycoforms at the glycopeptide level.**
- **For cases where high energy (MS/MS) data results in identical spectra, implementing high resolution ion mobility is shown to be a powerful technique for differentiation and therefore accurate characterisation.**
- **The flexibility for data acquisition using the Cyclic™ IMS, allows profiling data to be initially collected prior to a more targeted analysis, using the multi-pass functionality of the cyclic device for greater structural elucidation.**

References

1. Watanabe et al., *Science*. **2020**; 369:330-333.
2. Zhou et al., *Glycobiology*. **2018**; 31:69-80.
3. Sanda et al., Anal Chem. **2021**; 93:2003-2009.

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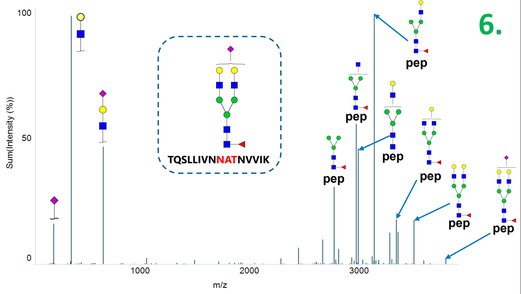

Figure 6 - High energy, deconvoluted spectrum for the intact glycopeptide TQSLLIVNATNVVIK (GlcNAc2Fuc1Man3GlcNAc2Gal2NeuAc1). Positional variation of the sialic acid linkage is the most probable explanation for the differences observed in drift time.

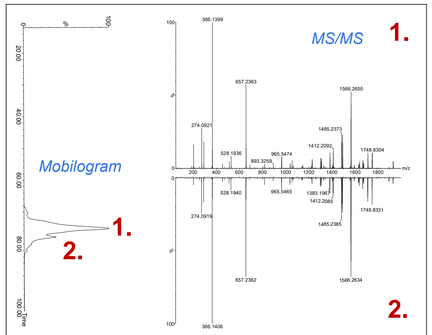

Figure 5 - 5 passes of the cyclic device indicates the presence of two potential glycoforms at different drift times (as shown on the mobilogram to the left), which relate to the peptide TQSLLIVNATNVVIK. The resulting MS/MS spectra for each drift time (mirror plot), are identical highlighting differentiation is only possible with implementing ion mobility at high resolution.

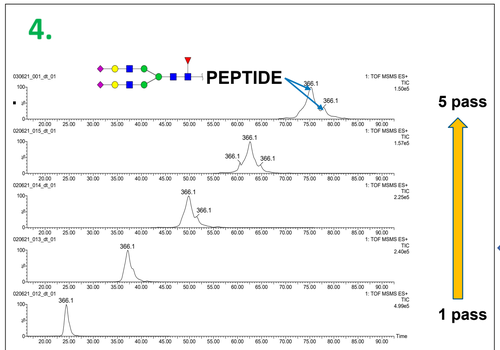

Figure 4 - Multiple passes of the mobility device, results in multiple glycoforms being highlighted as present. A single IMS pass (IMS resolution = 158 Ω/ΔΩ) shows a single peak, however, by 3 IMS passes a shoulder of the peak is evident, indicating the presence of additional glycoforms.

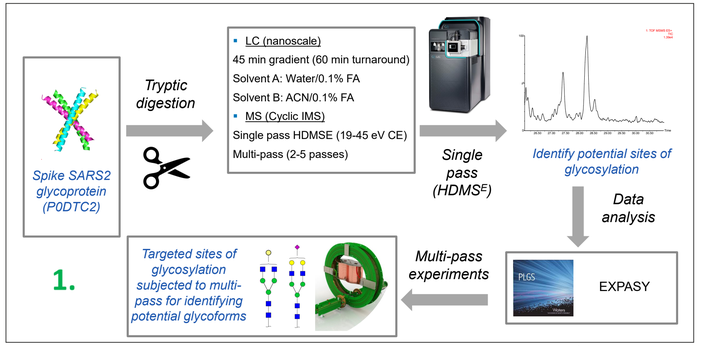

Figure 1 - Experimental design: Tryptic digestion followed by LC-MS analysis utilising the Cyclic™ ion mobility (cIMS) MS. Peptides were separated using 75 um chromatography over a 45 min gradient. MS data were collected as a single pass (HDMSE mode) and processed using ProteinLynx Global Server™ (PLGS). Based on the identification of candidate glycopeptides, targeted multi-pass experiments were performed to allow for differentiating between various glycoforms.

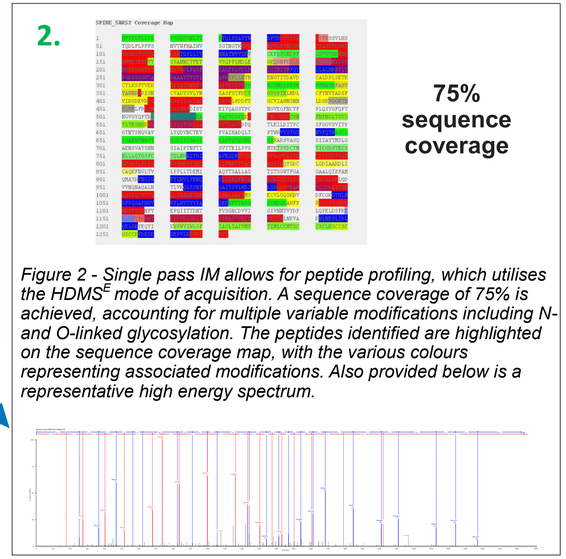

Figure 2 - Single pass IM allows for peptide profiling, which utilises the HDMSE mode of acquisition. A sequence coverage of 75% is achieved, accounting for multiple variable modifications including N- and O-linked glycosylation. The peptides identified are highlighted on the sequence coverage map, with the various colours representing associated modifications. Also provided below is a representative high energy spectrum.

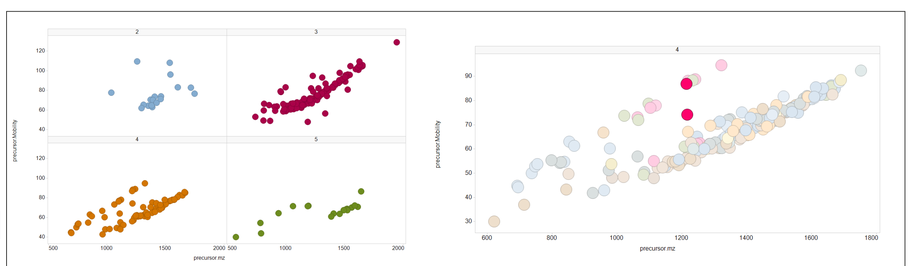

Figure 3 - The trellis above represents the distribution of N-linked glycopeptides according to m/z vs. mobility drift time. Each trellis corresponds with the peptide charge state, ranging from 2-5.

Focussing on the 4+ charge state, two different mobility trend lines are apparent (blue shaded areas). In particular, the peptide NHTSPDVDLGDISGINASVVNIQKEIDR exhibits two potential glycoforms of the same m/z but differ in drift time. Both forms are highlighted in red above.