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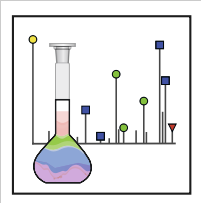

Highlights of Analytical Sciences in Switzerland

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A New Approach for Identifying Positional Isomers of Glycans Cleaved from Monoclonal Antibodies

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Keywords: Cryogenic infrared spectroscopy · Ion mobility separation · Positional isomers of glycans

Glycosylation patterns in monoclonal antibodies (mAbs) can vary significantly between different host cell types, and these differences may affect mAbs safety, efficacy, and immunogenicity. Recent studies have demonstrated that glycan isomers with the terminal galactose position on either the Man α1-3 or the Man α1-6 branch have an impact on the effector functions and dynamic structure of mAbs. One of the most powerful techniques for glycan investigation is the combination of liquid chromatography (LC) with mass spectrometry (MS), however, even this method cannot distinguish all the various forms of isomerism. The development of a robust technology to distinguish positional isomers of glycans is thus critical to guarantee mAb quality.

Our group has recently demonstrated that cryogenic infrared (IR at 45 K) spectroscopy provides unique vibrational spectra of glycans. Since spectroscopic fingerprints can be extremely sensitive to the slightest differences between molecules, we can distinguish all the various types of isomerism present in glycans. We apply the combination of IR spectroscopy with ultrahigh-resolution ion mobility separation (IMS) based on structures for lossless ion manipulation (SLIM) technology.

On the example of N-linked glycan G1F, we demonstrated the ability of our technique to assign the mobility-separated positional isomers (G1(α1-3)F and G1(α1-6)F) based on their unique IR fingerprint spectra. We then used these results to investigate the influence of the host cell line (CHO and HEK-293) on the G1F profile at the isomer level.

Our results demonstrate that the combination of high-resolution ion mobility and cryogenic ion spectroscopy provides a fast and reliable method for glycan isomer identification. It can be used to complement, or even replace, existing methods for establishing the similarity of glycan profiles between biological drugs and their biosimilars.

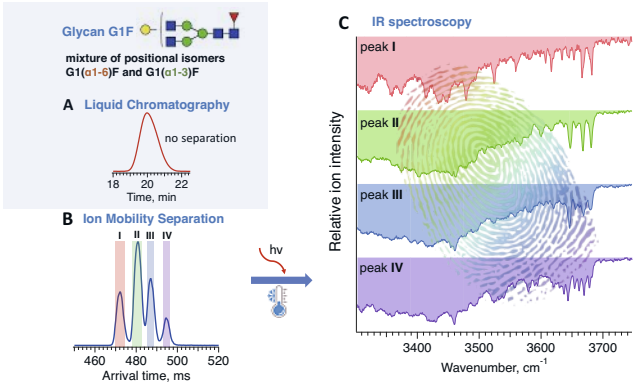

Fig. 1. Comparison of liquid chromatography (A) with our three-dimensional IMS-MS-IR approach (B, C) on the example of the N-linked glycan G1F which consists of nine monosaccharide units. Yellow circle: galactose; green circle: mannose; blue square: N-acetylglucosamine, red triangle: fucose.

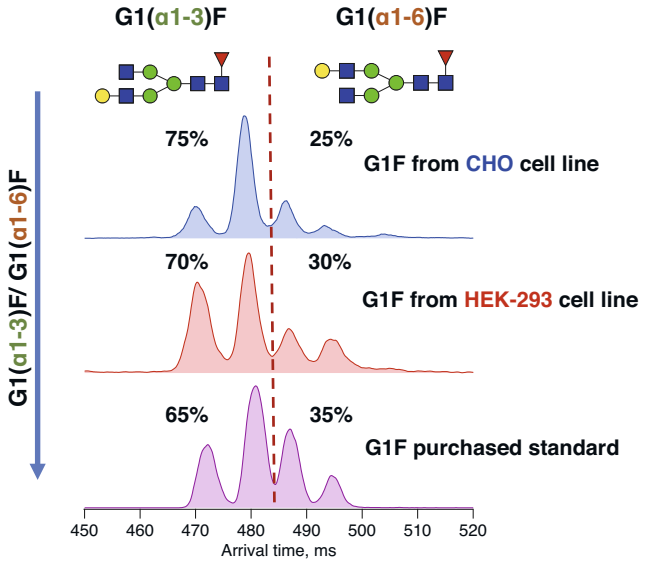

Fig. 2. Arrival time distributions of a G1F standard and G1F released from IgG antibody produced in CHO and HEK-293 cell lines.

Reference

I. Dyukova, A. Ben Faleh, S. Warnke, N. Yalovenko, V. Yatsyna, P. Bansal, T. R. Rizzo, *Analyst* **2021**, *146*, 4789, https://doi.org/10.1039/D1AN00780G.

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