SUPPORTING INFORMATION

Toward High-Throughput Cryogenic IR Fingerprinting of Mobility-Separated Glycan Isomers

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KEYWORDS: cryogenic IR spectroscopy, glycans, glycan analysis, glycomics, high throughput, ion mobility spectrometry

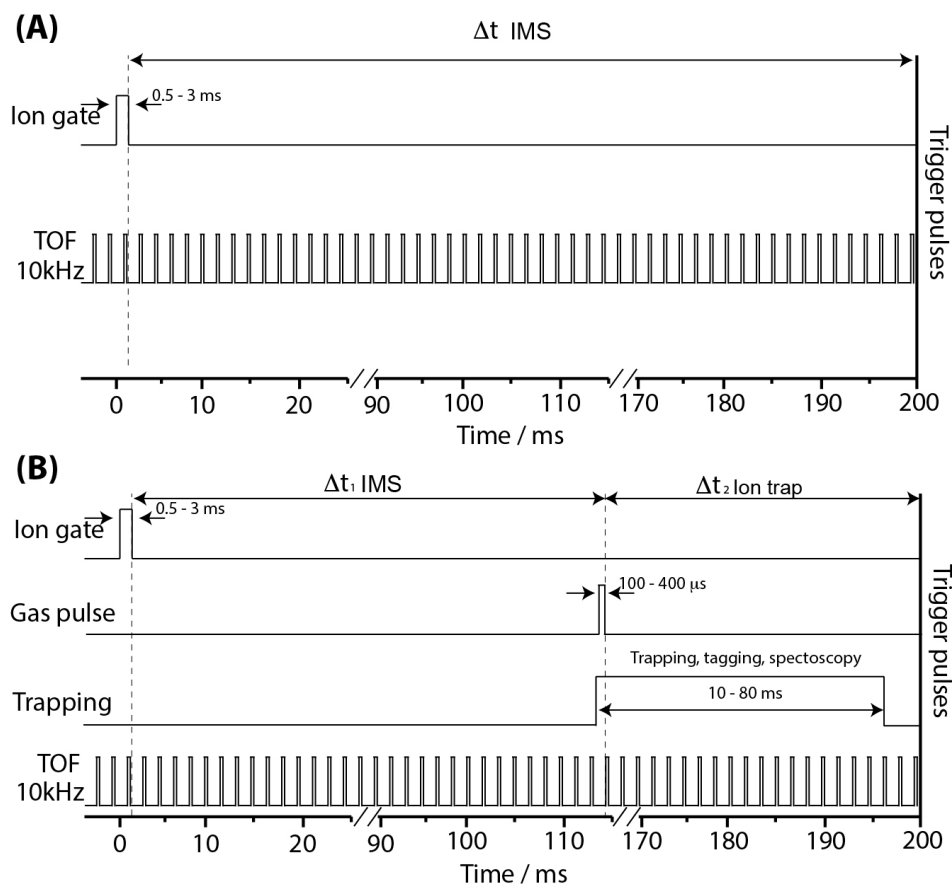**TYPICAL TIME SEQUENCE USED TO PERFORM THE EXPERIMENTS**

Figure S1: Typical time sequence used in SLIM IMS messenger tagging IR spectroscopy experiment using the newly developed instrument. (A) Time sequence diagram when running the instrument in IMS mode only. Ion are separated according to their mobility during almost the whole cycle length. The TOF acquires periodically at a frequency of 10kHz. (B) Time sequence diagram when running in IR spectroscopy mode. Δt_1 denotes the separation time on the SLIM module that can be adapted depending upon the considered molecule and the number of separation cycles. The trapping time during

which the ions are tagged and interrogated spectroscopically is denoted by Δt_2 and is in the range of tens of milliseconds. The TOF extraction frequency is not drawn to scale.

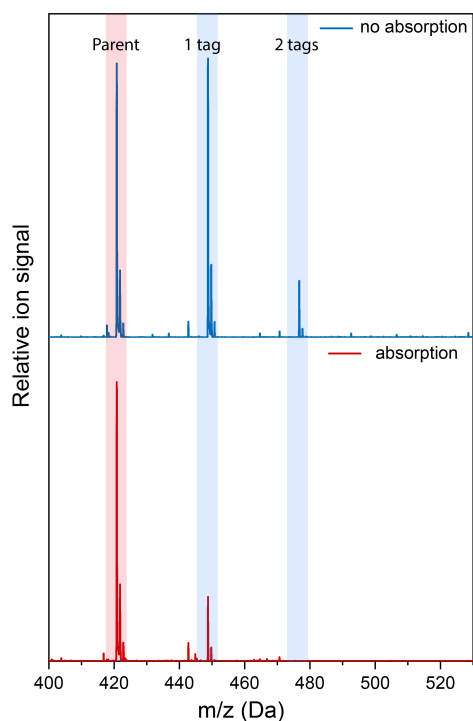## MASS SPECTRUM DISPLAYING THE TAGS DISTRIBUTION OF A DISACCHARIDE: ABSORPTION VS. NO ABSORPTION

Figure S2: Mass spectra of the sodiated GalNac-α(1-3)Gal ions when a resonant photon is absorbed (bottom panel) and when no photon is absorbed (top panel). The peak corresponding to the bare molecules is shown in red and the peaks corresponding the singly tagged ions in blue.

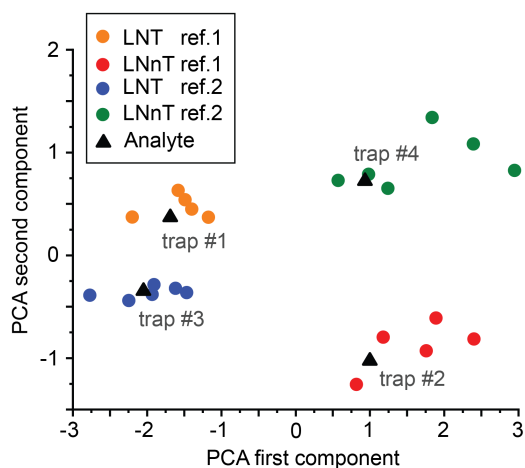## PCA ANALYSIS WITH REDUCED WAVENUMBER RANGE

Figure S3: First and second principal component after PCA of the IR fingerprint spectra shown in Fig. 4 in the main manuscript. PCA was applied over a reduced wavenumber range of 100 cm^{-1}, from 3600 cm^{-1} to 3700 cm^{-1}. The algorithm automatically groups spectra of the same compound and accurately assigns fingerprints obtained from the four different trap compartments to the reference data.