Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information for:

Unraveling the Isomeric Heterogeneity of Glycans: Ion Mobility Separations in Structures for Lossless Ion Manipulations

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Experimental Conditions and Instrument Parameters

All standards were purchased from Sigma-Aldrich (Milwaukee, WI USA), Megazyme (Wicklow, Ireland), or United States Biological Corp. (Swampscott, MA USA) without any further purification. All glycan standards were prepared to final concentrations of 5 μM in 50/50 HPLC grade water/methanol (*v/v*) with 0.5% acetic acid (*v/v*).

Details on the SLIM SUPER IM-MS platform used in these experiments has been described elsewhere [1-7]. Direct infusion was performed at flow rates of 300 nL/min with 3000 V nanoelectrospray voltage and 110 °C heated capillary. Traveling wave speed was kept at 320 m/s and traveling wave amplitude was optimized based on the observed mobilities for each glycan species for all experiments (shown in figure captions). SLIM chamber pressure was kept at 3.56 torr of helium gas and the ion funnel trap was kept at 3.50 torr. Data was acquired via homebuilt acquisition software, and an Agilent 6224 TOF mass spectrometer (Agilent Technologies, Santa Clara, CA) was used. Ions were accumulated for 1 s in-SLIM as described in detail below.

Ion Accumulation in the SLIM Device

As opposed to ion introduction via the ion funnel trap, ions can, instead, be accumulated in the SLIM device, itself. By halting the traveling wave in the second region (green region in Figure S1), a 'wall' is created so that the blue region functions as a giant accumulation space. Consequently, this wall also acts in lieu of any needs for compression ratio ion mobility programming (CRIMP), since ions are halted at the blue-green interface prior to separation. This in turn will increase both the resolution of measurements (initial peak width can be compressed via the 'wall') as well as sensitivity (1 second of in-SLIM accumulation introduces 100-1000 more ions than via the ion funnel trap).

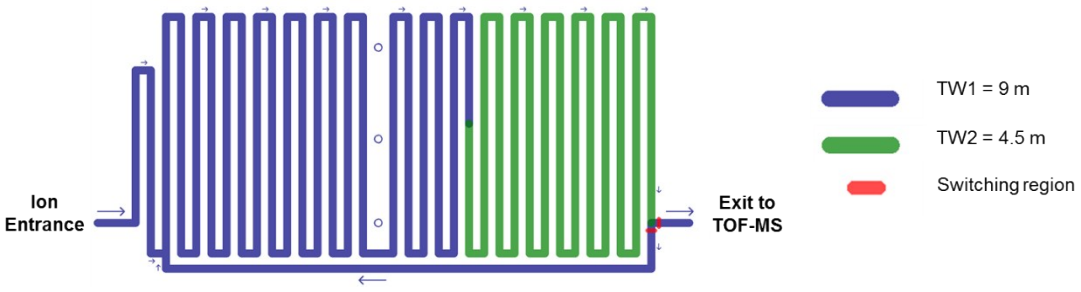

Figure S1. Diagram of SLIM SUPER module used in all experiments.

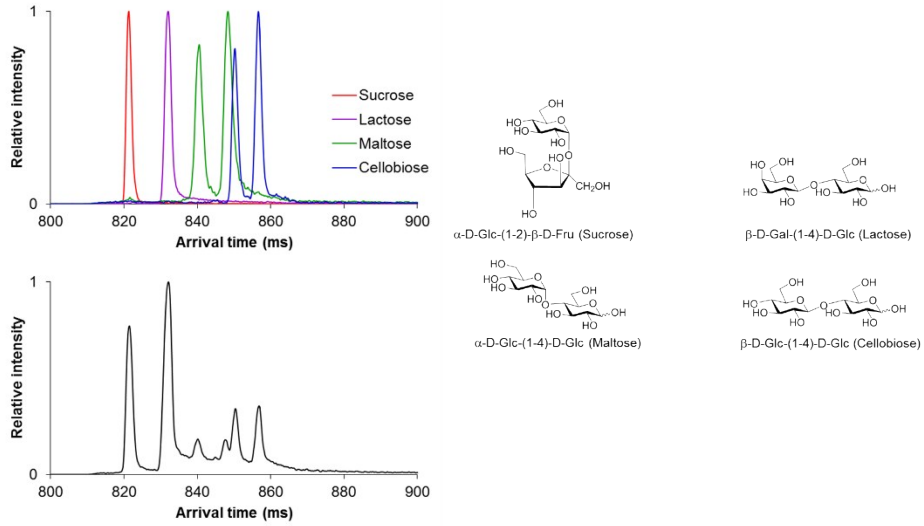

Figure S2. 112.5 m SLIM SUPER IM separations at 20 V traveling wave amplitude of isomeric disaccharides, 365.1 *m/z* $[M+Na]^+$, both as a mixture and as their individual standards.

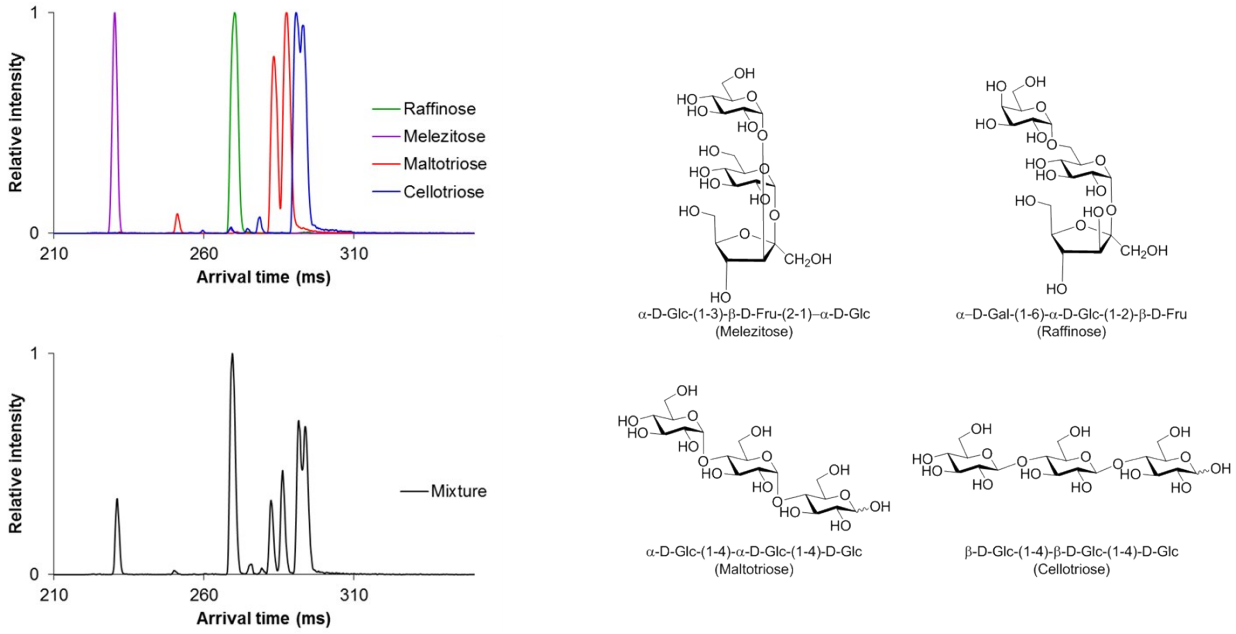

Figure S3. 45 m SLIM SUPER IM separations at 30 V traveling wave amplitude of isomeric trisaccharides, 527.2 *m/z* $[M+Na]^+$, both as a mixture and as their individual standards.

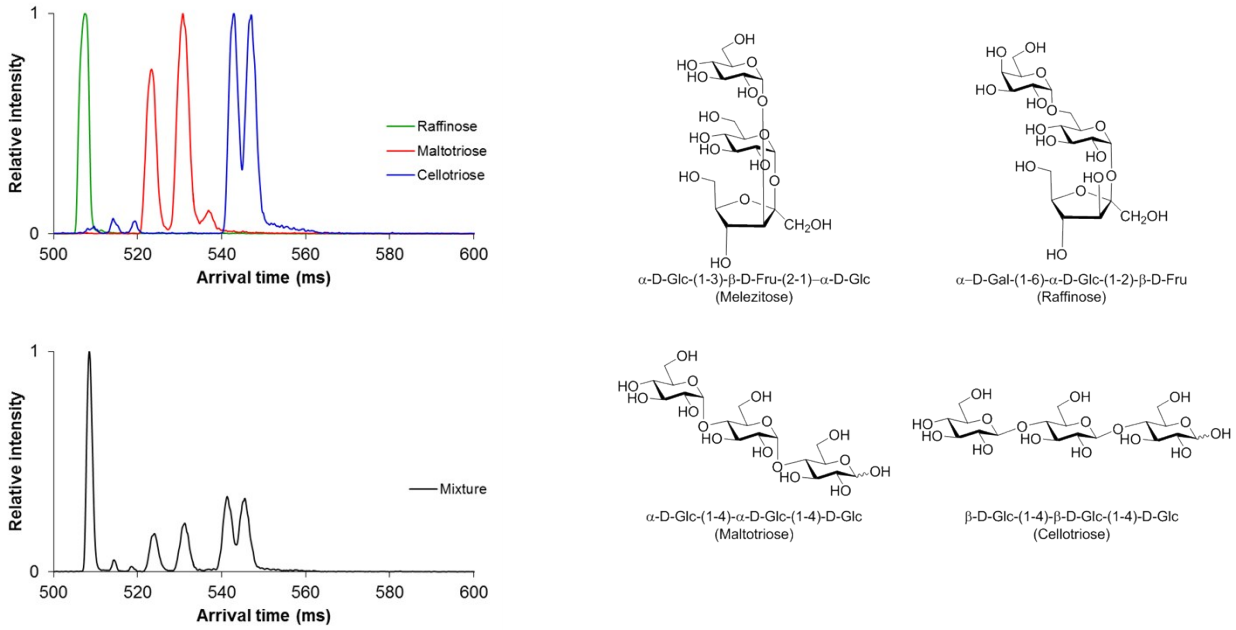

Figure S4. 85.5 m SLIM SUPER IM separation at 30 V traveling wave amplitude of the same isomeric trisaccharides, 527.2 *m/z* [M+Na^+] showing increased resolution of the substructures of maltotriose and cellotriose as compared to Figure S2.

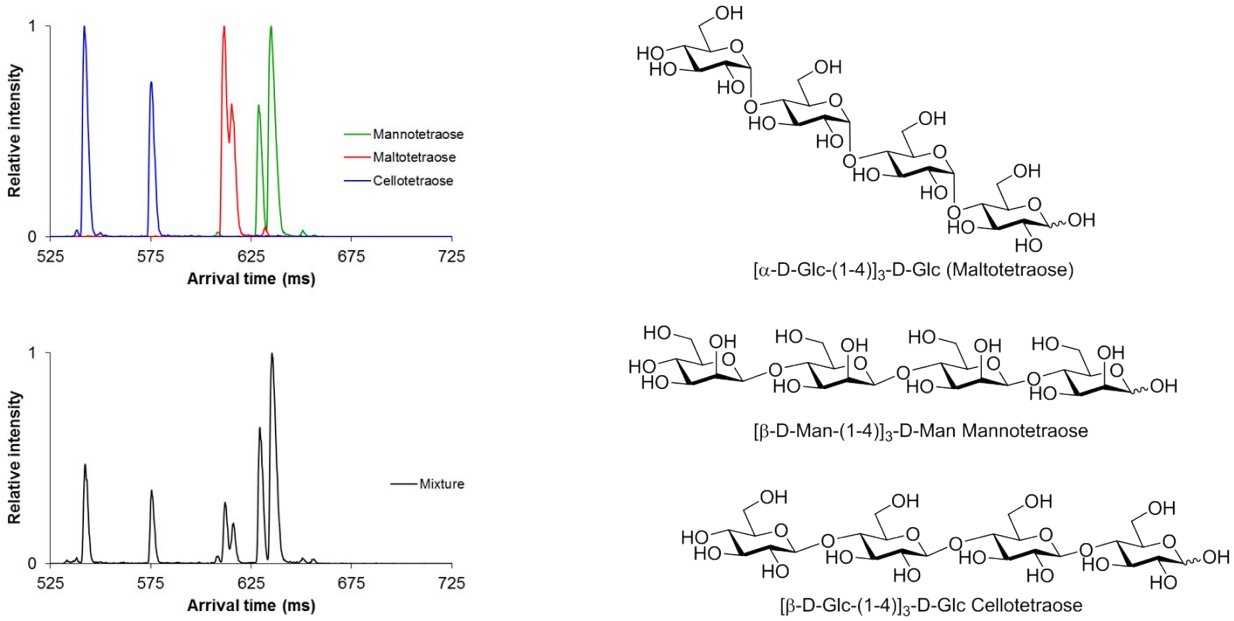

Figure S5. 72 m SLIM SUPER IM separations at 30 V traveling wave amplitude of isomeric trisaccharides, 689.2 *m/z* $[M+Na]^+$, both as a mixture and as their individual standards.

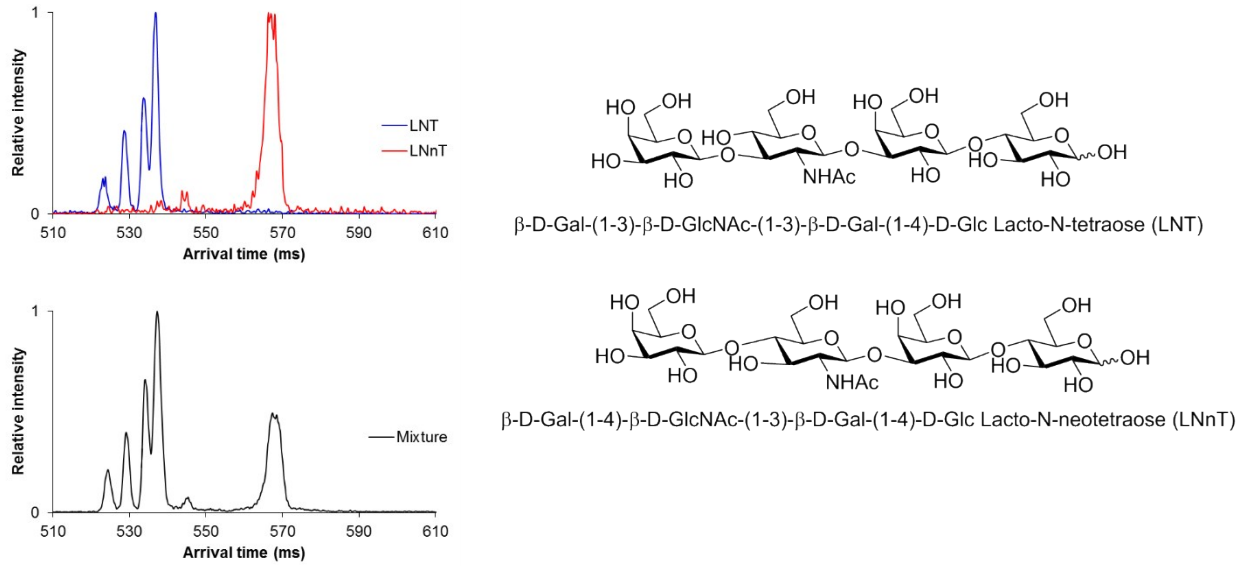

Figure S6. 31.5 m SLIM SUPER IM separations at 25 V traveling wave amplitude of LNT/LNnT isomers, 708.3 *m/z* $[M+H]^+$, both as a mixture and as their individual components.

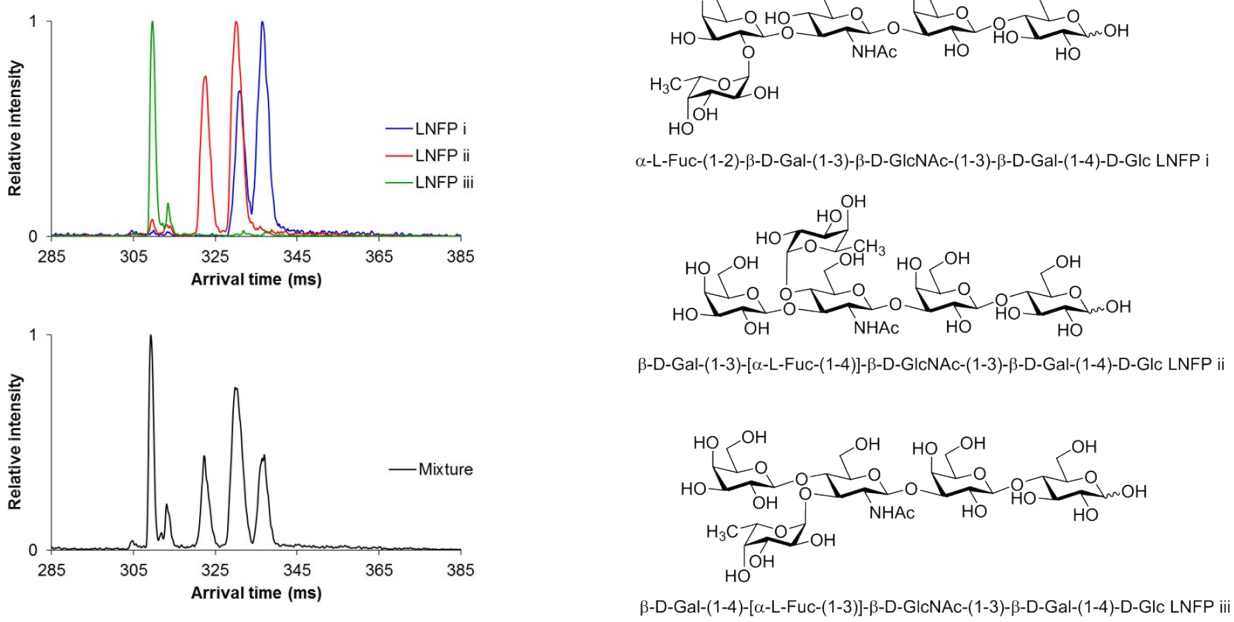

Figure S7. 45 m SLIM SUPER IM separations at 20 V traveling wave amplitude of LNFP isomers, 446.6 *m/z* $[M+H+K]^{2+}$, both as a mixture and as their individual components.

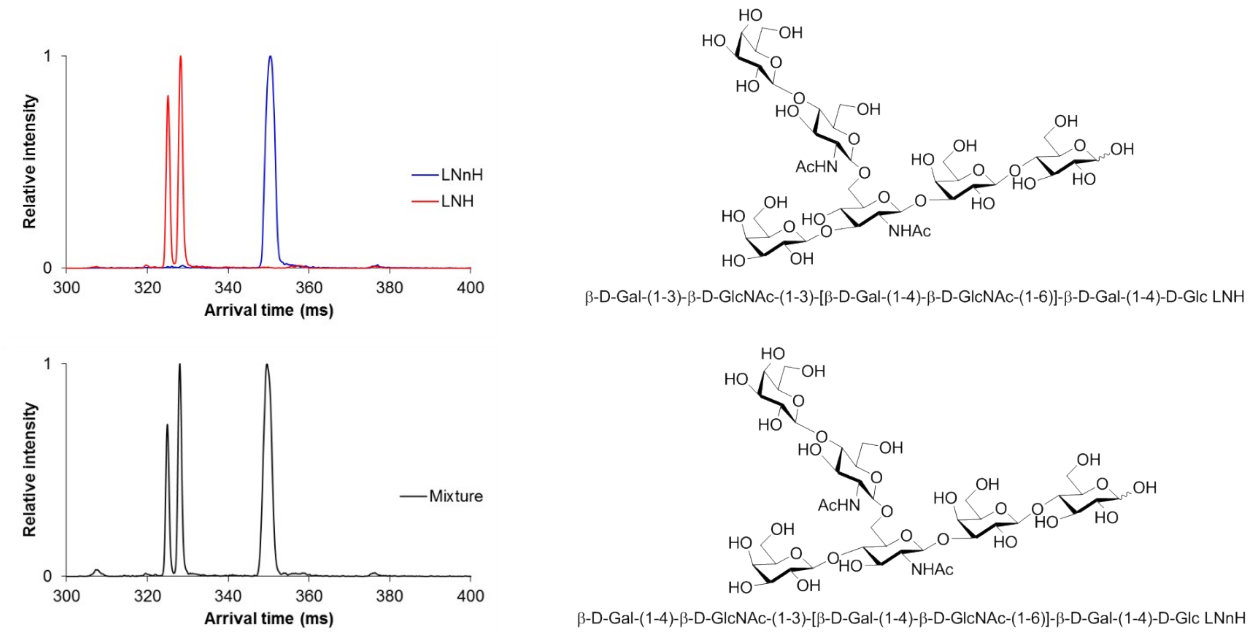

Figure S8. 58.5 m SLIM SUPER IM separations at 25 V traveling wave amplitude of LNH/LNnH isomers, 559.2 *m/z* $[M+2Na]^{2+}$, both as a mixture and as their individual components. While these LNH/LNnH isomers have been previously separated in our group [3], these new results demonstrate that superior separation can be achieved as their doubly sodiated adducts and in helium drift gas, instead of nitrogen.

1. L. Deng, S. V. B. Garimella, A. M. Hamid, I. K. Webb, I. K. Attah, R. V. Norheim, S. A. Prost, X. Zheng, J. A. Sandoval, E. S. Baker, Y. M. Ibrahim and R. D. Smith, *Anal. Chem.* , 2017, **89**, 6432-6439.
2. L. Deng, Y. M. Ibrahim, E. S. Baker, N. A. Aly, A. M. Hamid, X. Xhang, X. Zheng, S. V. B. Garimella, I. K. Webb, S. A. Prost, J. A. Sandoval, R. V. Norheim, G. A. Anderson, A. V. Tolmachev and R. D. Smith, *ChemistrySelect*, 2016, **1**, 2396-2399.
3. L. Deng, I. K. Webb, S. V. B. Garimella, A. M. Hamid, X. Zheng, R. V. Norheim, S. A. Prost, G. A. Anderson, J. A. Sandoval, E. S. Baker, Y. M. Ibrahim and R. D. Smith, *Anal. Chem.* , 2017, **89**, 4628-4634.
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5. L. Deng, Y. M. Ibrahim, A. M. Hamid, S. V. Garimella, I. K. Webb, X. Zheng, S. A. Prost, J. A. Sandoval, R. V. Norheim, G. A. Anderson, A. V. Tolmachev, E. S. Baker and R. D. Smith, *Anal. Chem.* , 2016, **88**, 8957-8964.
6. C. D. Chouinard, G. Nagy, I. K. Webb, S. V. B. Garimella, E. S. Baker, Y. M. Ibrahim and R. D. Smith, *Anal. Chem.*, 2018, DOI: 10.1021/acs.analchem.8b02990.
7. C. D. Chouinard, G. Nagy, I. K. Webb, T. Shi, E. S. Baker, S. A. Prost, T. Liu, Y. M. Ibrahim and R. D. Smith, *Anal. Chem.*, 2018, DOI: 10.1021/acs.analchem.8b02397.