Supporting Information 1

Dopant-enriched nitrogen gas for enhanced electrospray ionization of released glycans in negative ion mode

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S1. Materials and methods

N- and *O*-glycan release from purified glycoproteins

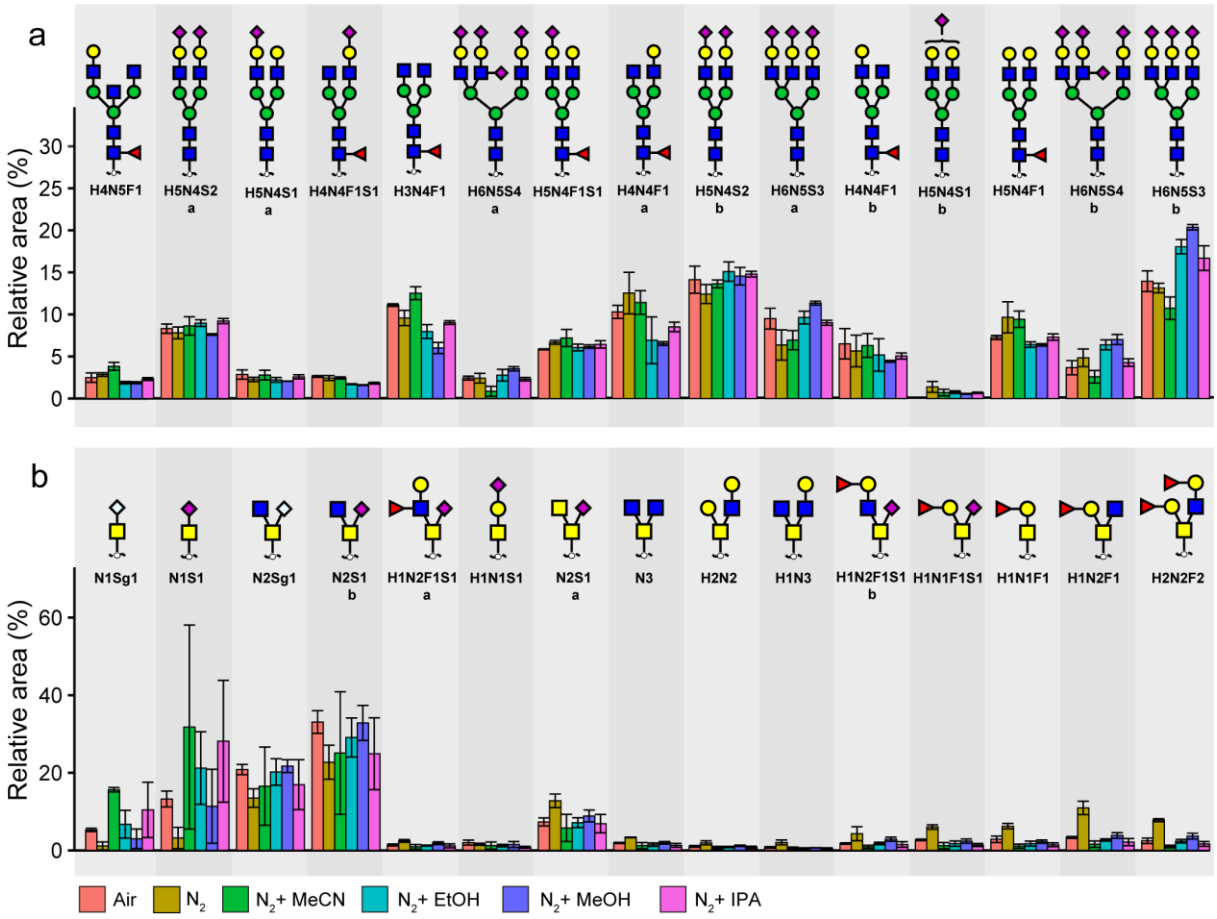
Twenty µg of purified glycoproteins IgG, bovine fetuin and ten µg of bovine submaxillary mucin were blotted onto separate wells of preconditioned hydrophobic Immobilon-P PVDF membrane. Both N- and O-glycans were released from the glycoproteins as described before [13]. Briefly, *N*-glycans were released by PNGase F digestion overnight at 37°C. Upon recovery of the released *N*-glycans, *O*-glycan release was performed via reductive beta-elimination (0.5M $NaBH_4$ in 50 mM KOH at 50°C for 16 hours). The recovered *N*-glycans were reduced by 1M $NaBH_4$ in 50 mM KOH at 50°C for 3 hours. Desalting of the released *N*- and *O*-glycan samples was performed using cation exchange resin Dowex 50W X8 in 96-well filter plates. Finally, the desalted *N*- and *O*-glycans were further purified using graphitized carbon solid phase extraction in 96-well filter plates. Samples were stored at -20˚C before analysis.

Measurements with PGC-LC-MS/MS using different dopant solvents

A custom-made trap column (size 30 × 0.32 mm) packed with 5 µm particle size PGC stationary phase from Hypercarb PGC analytical column (size 100 × 4.6 mm, 5 µm particle size, Thermo Fisher Scientific, Waltham, MA) was used to load the samples using 100% buffer A (10 mM ammonium bicarbonate) at a loading flow of 6 µL/min. The glycans were separated on a custom-made PGC column (100 mm × 100 µm, 3 µm particle size obtained from Thermo Fisher Scientific) at a 0.6 µL/min flow rate by applying a linear gradient from 1% to 50% buffer B (MeCN, 10 mM ammonium bicarbonate) over 73 min for *O*-glycans and a multi-step gradient 2–9% buffer B in 1 min and 9% to 40.5% buffer B in 70 min for *N*-glycans. A constant column temperature of 45˚C was maintained. The LC system was coupled to an amaZon ETD speed ESI ion trap MS using the CaptiveSpray™ source (Bruker Daltonics) with an applied capillary voltage of 1000 V in negative-ionization mode. The drying gas (N_2) temperature was set at 280˚C and the flow to 3 L/min. The nebulizer gas pressure was kept at 3 psi (for all conditions except for ambient air). The nanoBooster™ bottle (Bruker Daltonics) was filled with different dopant solvents, namely MeOH, EtOH, IPA and MeCN. The measurements were performed by filling the bottle with 800 mL solvent, and the level of the dopant inside the bottle was never below 500 mL. MS spectra were acquired in enhanced mode within a mass to charge ratio (*m/z*) range of 380-1850, target mass of smart parameter setting was set to *m/z* 800 (*O*-glycans) or *m/z* 1000 (*N*-glycans); ion charge control (ICC) to 40,000 and maximum acquisition time to 200 ms. MS/MS spectra were generated by collision-induced dissociation on the three most abundant precursors, applying an isolation width of 3 Th. The fragmentation cut-off was set to 27% with 100% fragmentation amplitude using the Enhanced SmartFrag option (30–120% in 32 ms) and ICC was set to 150 000. For quality control bovine fetuin released *N*-glycan standards were used to monitor instrument performance. Extracted ion chromatograms including all observed charge states (1, 2 and 3) of the first 3 isotopes were used to integrate the area under the curve (AUC) for each individual glycan isomer using Compass DataAnalysis software v.5.0. Peaks were manually picked and integrated. Selected glycan peaks were confirmed by MS/MS and by comparison with literature reports [14–18].Relative quantitation was performed using the total area of all glycans within one sample as reference (100%). Data analysis and visualization was performed using in-house developed "R'' scripts.

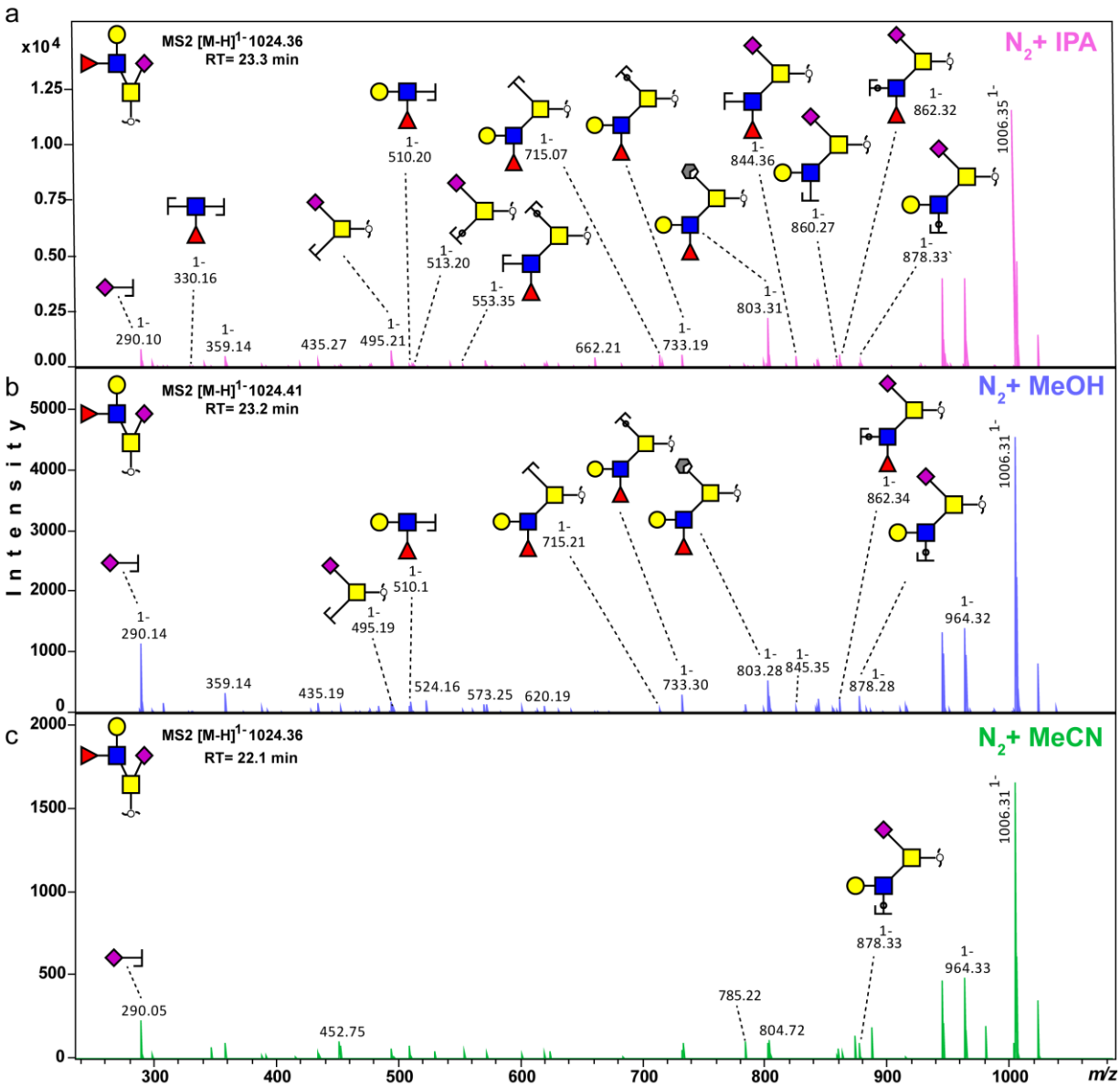
S2 . Figures

Figure S1



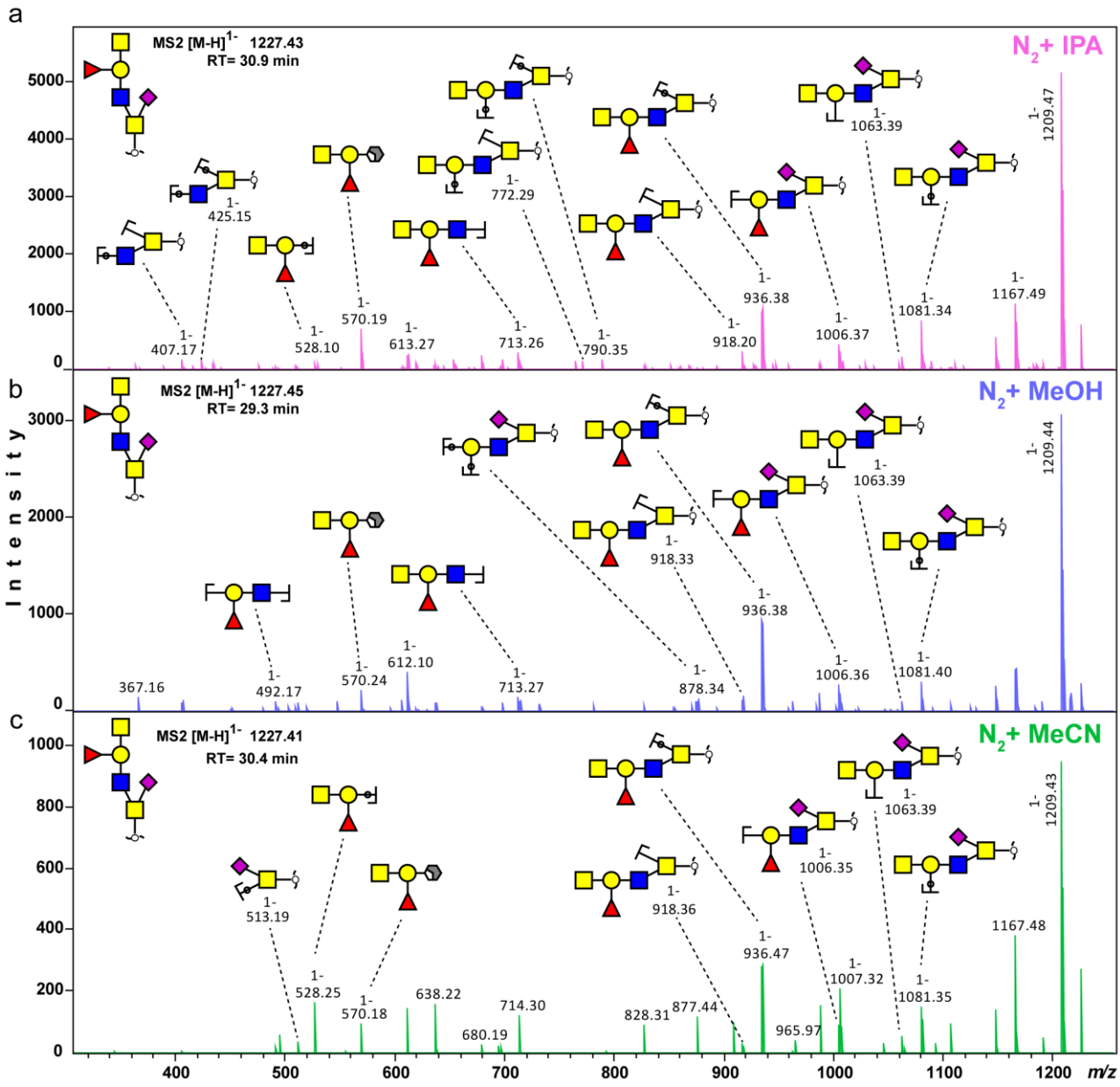
Supplementary Figure S2: Effect of different dopant solvents on the relative areas of different glycans. Relative areas of **(a)** *N*-glycans released from IgG and Bovine fetuin and (**b)** *O*-glycans released from from Bovine submaxillary mucin

Figure S2



Supplementary Figure S3: Effect of different dopant solvents on the fragment spectrum intensities of early eluting O-glycans. Tandem MS spectrum of *O*-glycan with composition H1N1F1S1 with **(A)** IPA, **(B)** MeOH and **(C)** MeCN. Peaks with intensity below 100 are not labelled and annotated.

Figure S3



Supplementary Figure S4: Effect of different dopant solvents on the fragment spectrum intensities of early eluting O-glycans. Tandem MS spectrum of an *O*-glycan with composition H1N3F1S1 with **(A)** IPA, **(B)** MeOH and **(C)** MeCN. Peaks with intensity below 100 are not labelled and annotated.