Deciphering the composite GlycA/B inflammation markers in NMR spectra of human blood

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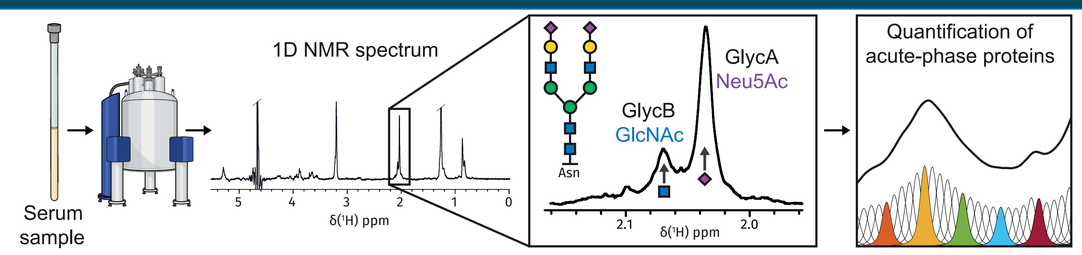
Diagnostic potential of NMR glycan signals for quantification of acute-phase proteins



NMR spectra of human serum and plasma offer valuable insights into the physiological status of individuals by revealing signals arising from metabolites, lipoproteins, and notably, glycoproteins. So far, the glycoprotein NMR signatures have not been well characterized despite holding substantial diagnostic value due to the vital role of serum glycoproteins in health and disease. Only the two most intense glycoprotein signals, termed GlycA and GlycB, have been shown to be good biomarkers for cardiovascular diseases and several inflammatory conditions. Interestingly, our recent findings demonstrate that specific features in 1D NMR spectra can be associated with individual acute-phase inflammatory proteins, enabling rapid quantification of glycoproteins by NMR. [1]

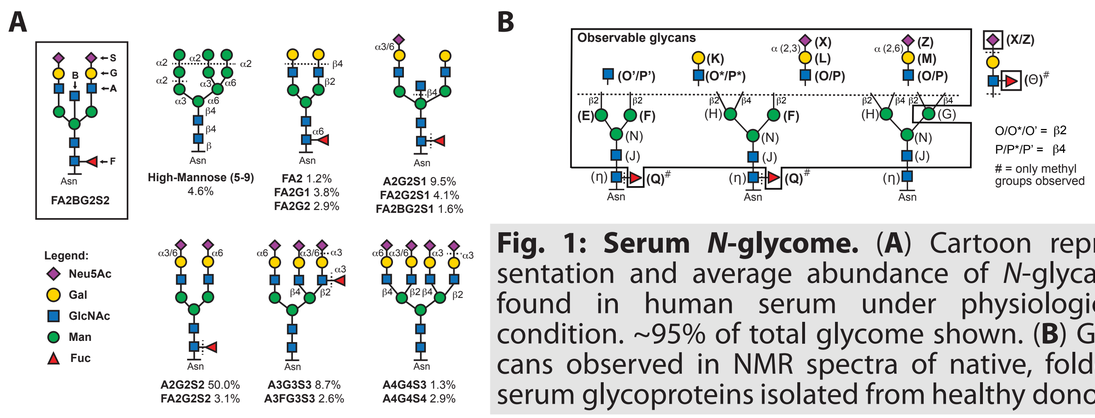


Fig. 1: Serum *N*-glycome. (**A**) Cartoon representation and average abundance of *N*-glycans found in human serum under physiological condition. ~95% of total glycome shown. (**B**) Glycans observed in NMR spectra of native, folded serum glycoproteins isolated from healthy donors.

Serum glycoproteins bear a plethora of different glycans giving rise to diverse composite NMR signatures. Upon inflammatory and other pathological processes, not only are protein concentrations modulated, but also the glycosylation profiles change, offering diagnostic potential. To explore this, we pursued to parallel strategies: (i) Isolation and characterization of individual, native glycoproteins from human serum and (ii) examination of differences in glycoprotein signatures for distinct pathological conditions.

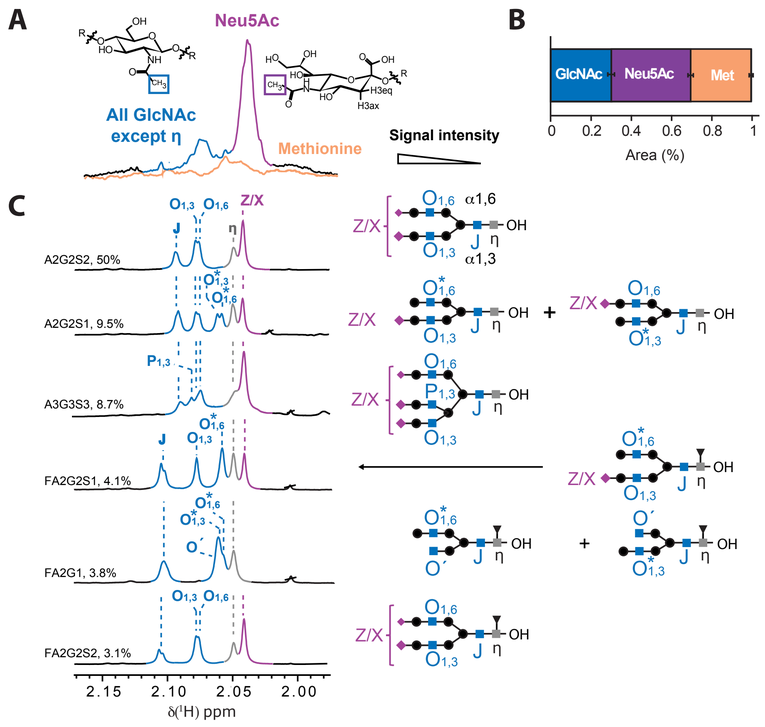


Fig. 2: Decomposition of the GlycA/B signals. [1] (**A**) Composition of the GlcNAc and Neu5Ac *N*-acetyl signals of a healthy control measured with a JEDI-PGPE experiment [2]. (**B**) Relative signal contributions obtained from consecutive enzymatic digestions with neuraminidase and PNGase F. (**C**) 1D NMR spectra of the *N*-acetyl methyls of *N*-glycan standards corresponding to the most abundant glycans in human plasma (~79% of the *N*-glycans normally found in human plasma).

Isolation and characterisation of native glycoproteins from serum

Isolation strategy

I. Ultracentrifugation → **II.** DEAE-Affi chromatography → **III.** Size-exclusion chromatography

To isolate individual serum glycoproteins, lipoproteins were first removed by flotation ultracentrifugation (I.) [3]. Glycoproteins were then fractionated by bifunctional affinity/ion-exchange chromatography (DEAE-Affi, II.) [4], followed by size-exclusion chromatography (III.) which was repeated if required. Proteins were characterized by native and SDS-PAGEs and Western blots.

1D NMR profiles reveal characteristic, distinct features

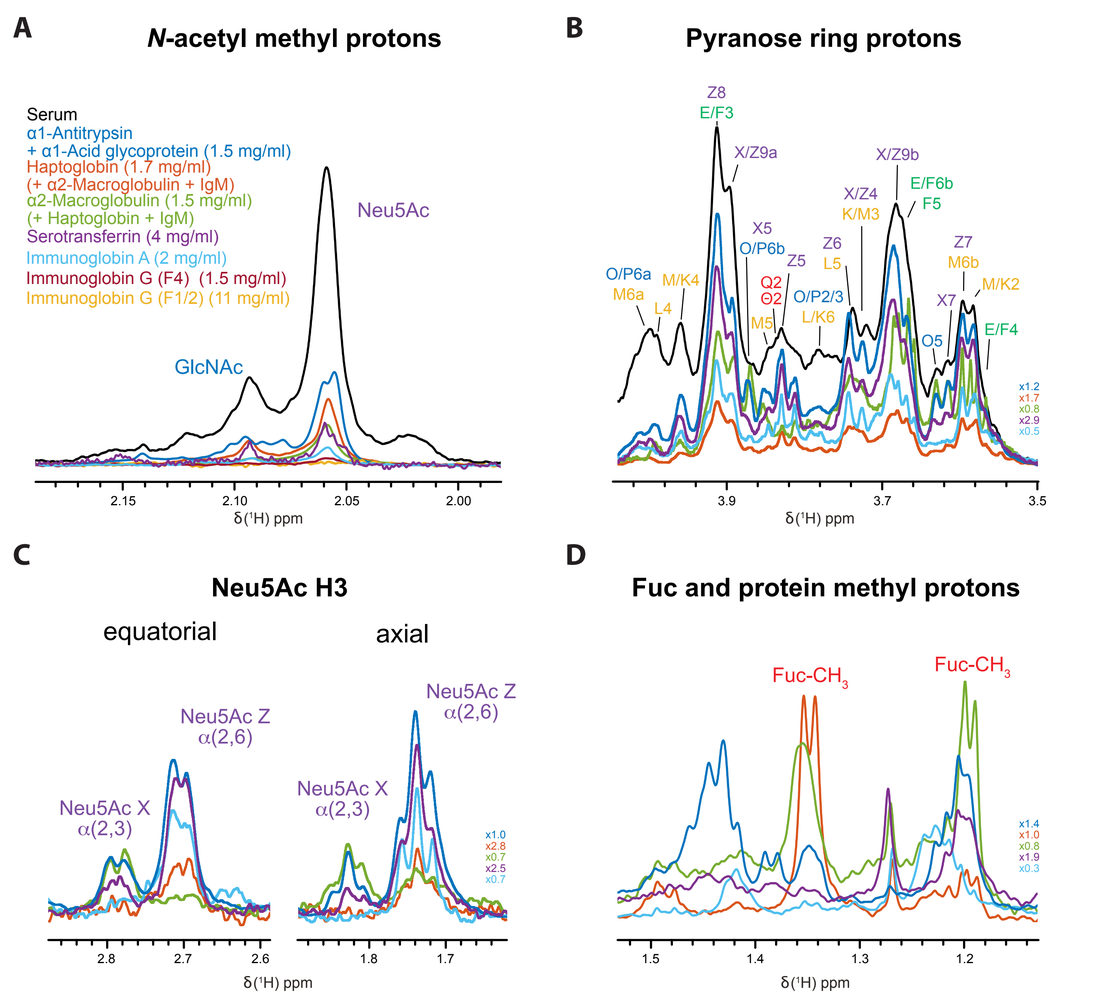


Fig. 3: 1D NMR spectra of isolated glycoproteins. (**A**) *J*-edited diffusion (JEDI) spectra of serum and isolated glycoproteins from the same healthy donor. Spectra are scaled to physiological concentrations to estimate the individual contributions to the overall *N*-acetyl glycan signals observed in serum. (**B**-**D**) Characteristic spectral regions of glycoproteins of interest in CPMG spectra with assignments.

Modulation of glycoprotein signatures in pathological conditions

Case study: Inflammatory bowel disease (IBD)

79 patients with acute IBD (Crohn's disease or Ulcerative colitis) + 50 healthy age-, sex- and BMI matched controls → 5 mm serum IVDr NMR samples [5] → Acquisition of 1D NMR spectra → Quantification of glycoprotein signals (for details see Poster P-257 and [1])

Glycoprotein signals as biomarkers for IBD

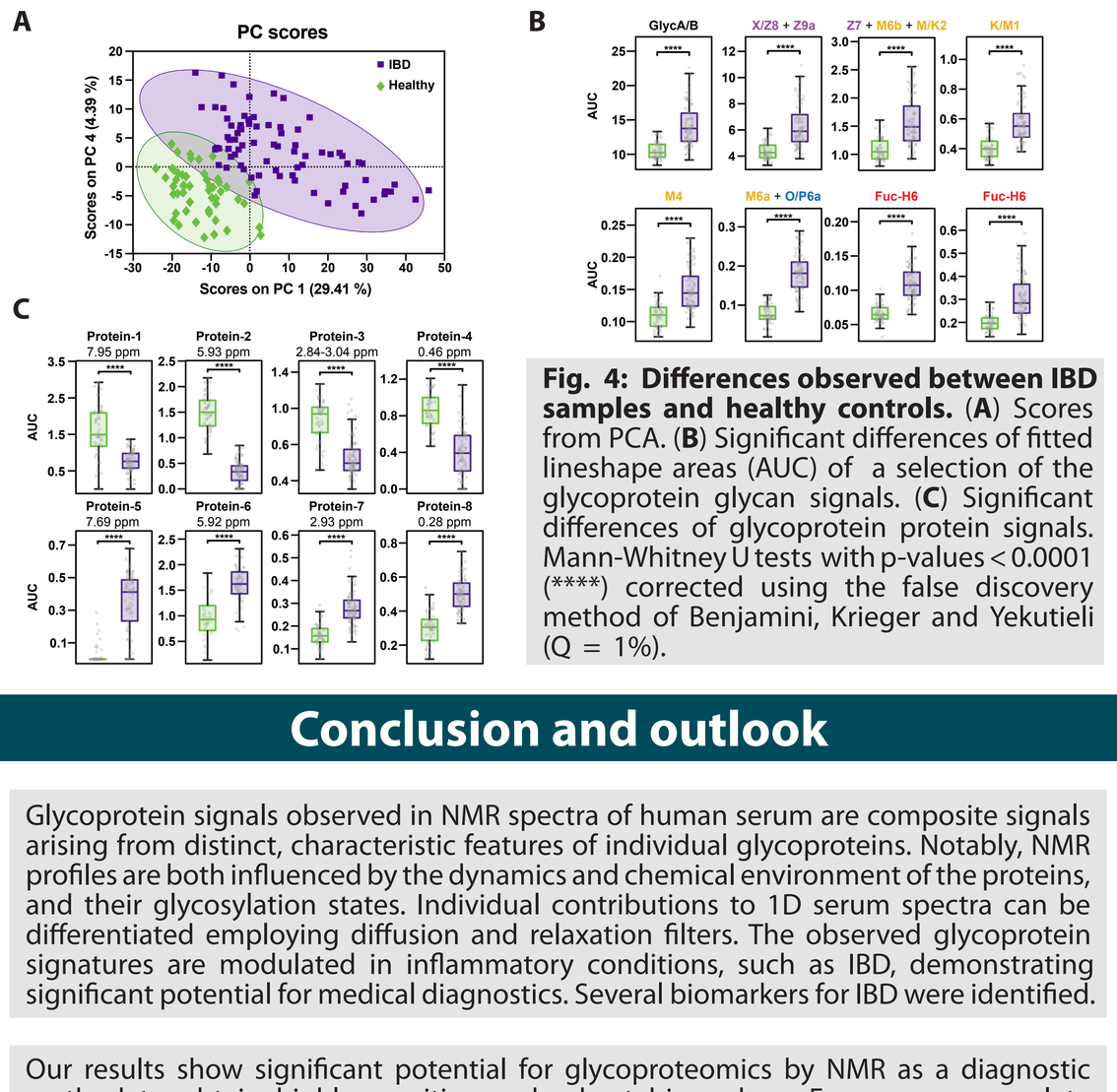


Fig. 4: Differences observed between IBD samples and healthy controls. (**A**) Scores from PCA. (**B**) Significant differences of fitted lineshape areas (AUC) of a selection of the glycoprotein glycan signals. (**C**) Significant differences of glycoprotein protein signals. Mann-Whitney U tests with p-values < 0.0001 (****) corrected using the false discovery method of Benjamini, Krieger and Yekutieli (Q = 1%).

Conclusion and outlook

Glycoprotein signals observed in NMR spectra of human serum are composite signals arising from distinct, characteristic features of individual glycoproteins. Notably, NMR profiles are both influenced by the dynamics and chemical environment of the proteins, and their glycosylation states. Individual contributions to 1D serum spectra can be differentiated employing diffusion and relaxation filters. The observed glycoprotein signatures are modulated in inflammatory conditions, such as IBD, demonstrating significant potential for medical diagnostics. Several biomarkers for IBD were identified.

Our results show significant potential for glycoproteomics by NMR as a diagnostic method to obtain highly sensitive and robust biomarkers. For a more complete assignment of acute-phase glycoproteins and their medical relevance, samples from different diseases will have to be analysed. Moving forward, our objective is to develop efficient, NMR-based diagnostic tools to derive biomarkers to diagnose and monitor different diseases based on specific glycoprotein markers.

References

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