MALDI-MS method for discovery of glycan biomarkers in colorectal cancer

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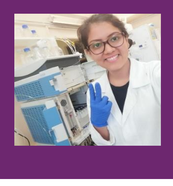

Introduction

Colorectal cancer (CRC) is the third most common cancer detected in both men and women and the second most common cause of cancer deaths in the United States according to the World Health Organization [1]. Some studies have identified presence of aberrant glycans at early stages of CRC and changes in the glycosylation pathway are associated with the CRC progression [2]. To establish a glycosylation analysis is still challenging because of the low throughput, sample preparation is very laborious and time consuming, the system is very complex to operate and highly qualified analyst is needed to interpret the mass spectrometry (MS) spectra [3]. Since glycans can be useful biomarkers for early detection of CRC is necessary to stablish a glycosylation analysis. In our study we are aiming to identify potential glycobiomarkers in model glycoproteins and serum samples using matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) MS.

Methodology

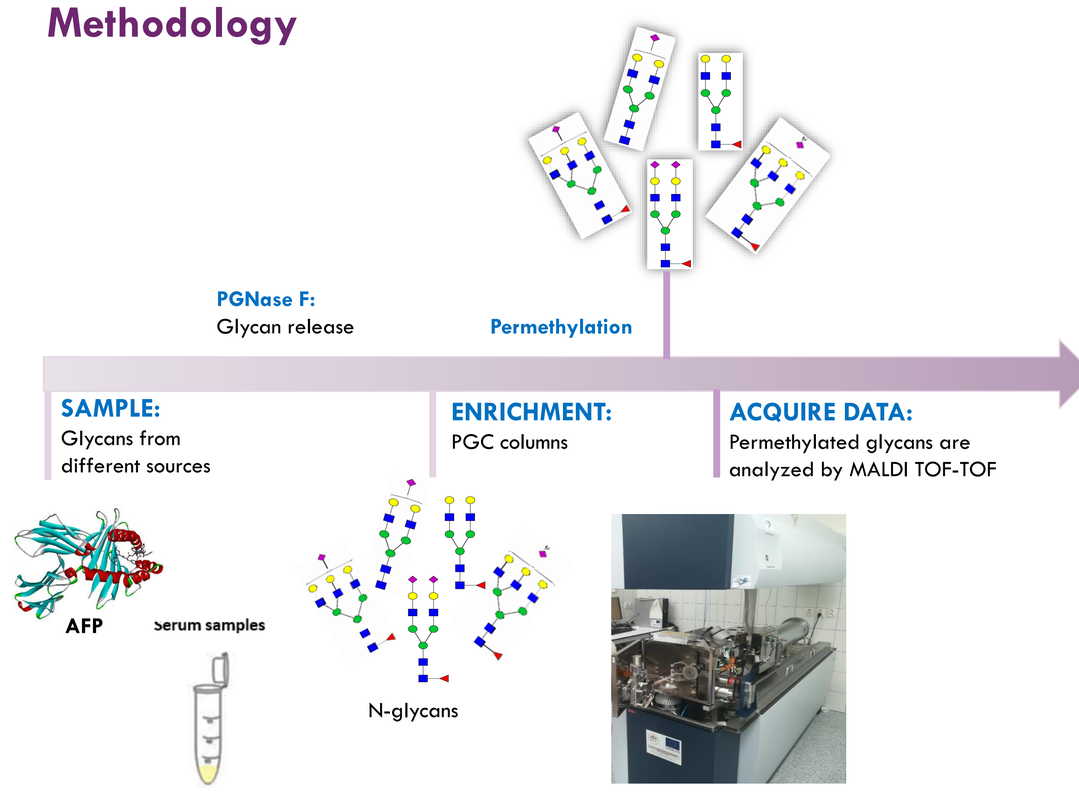

Fig 1. Workflow for glycan analysis analysis using mass spectrometry

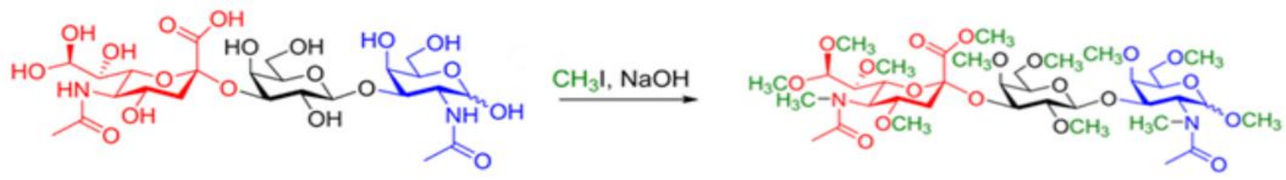

Fig 4. During permethylation, the methyl groups are added to hydroxyl and N-acetyl groups of N-glycans [4]

Results

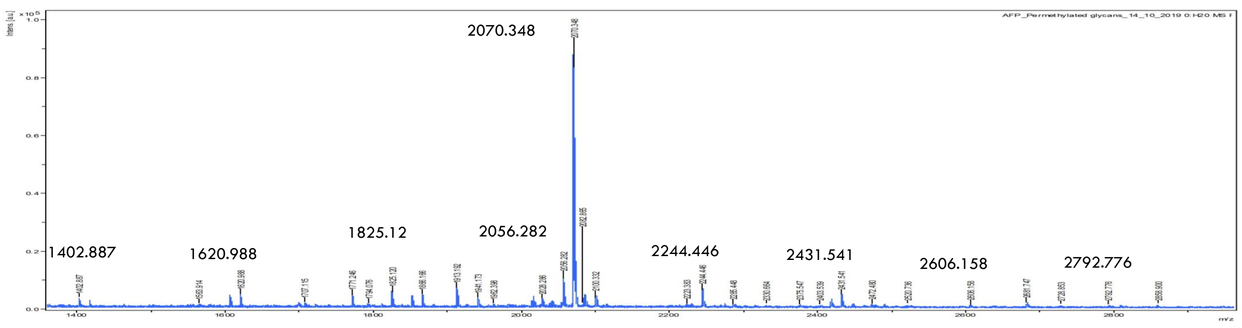

Fig 2. Permethylated N-glycans from AFP performed in positive ion mode m/z range 1000-4000

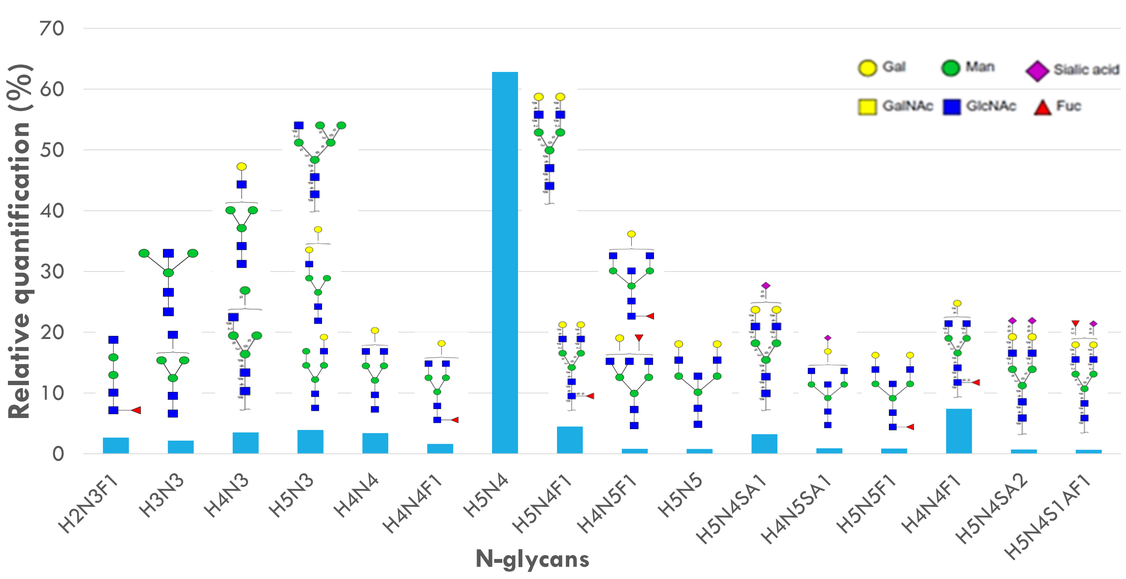

Fig 3. Relative abundance of N-glycans present in AFP

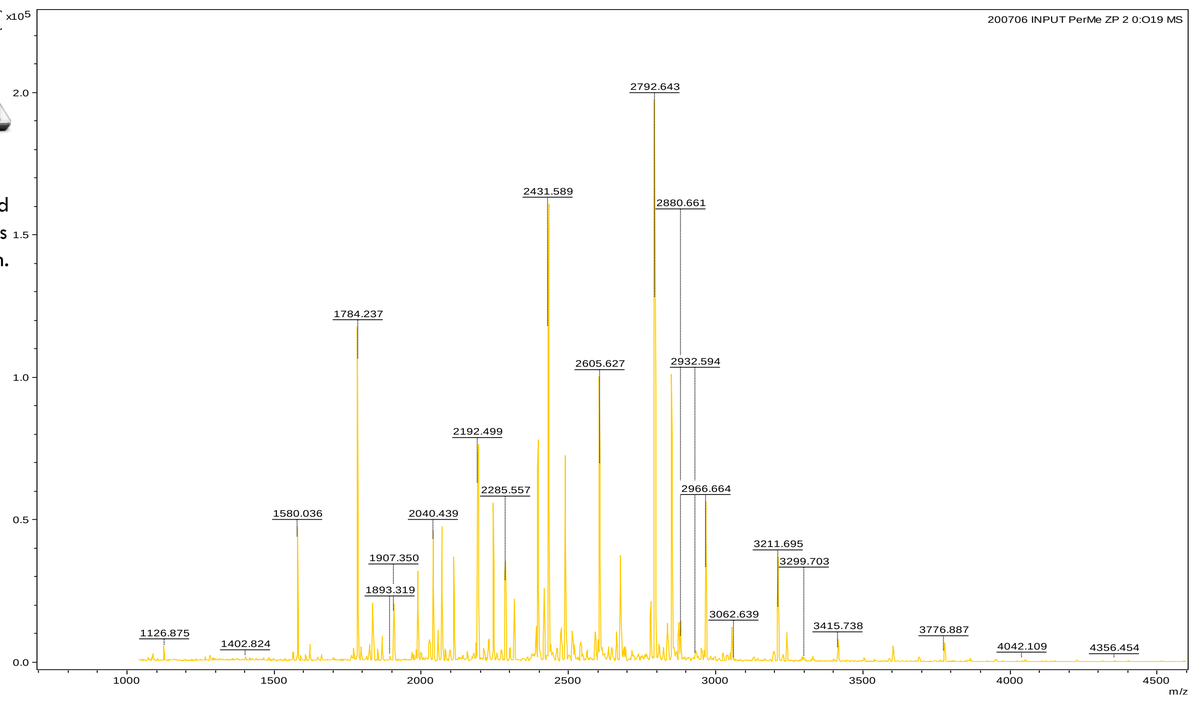

Fig 5. Permethylated N-glycans from serum performed in positive ion mode m/z range 1000-5000

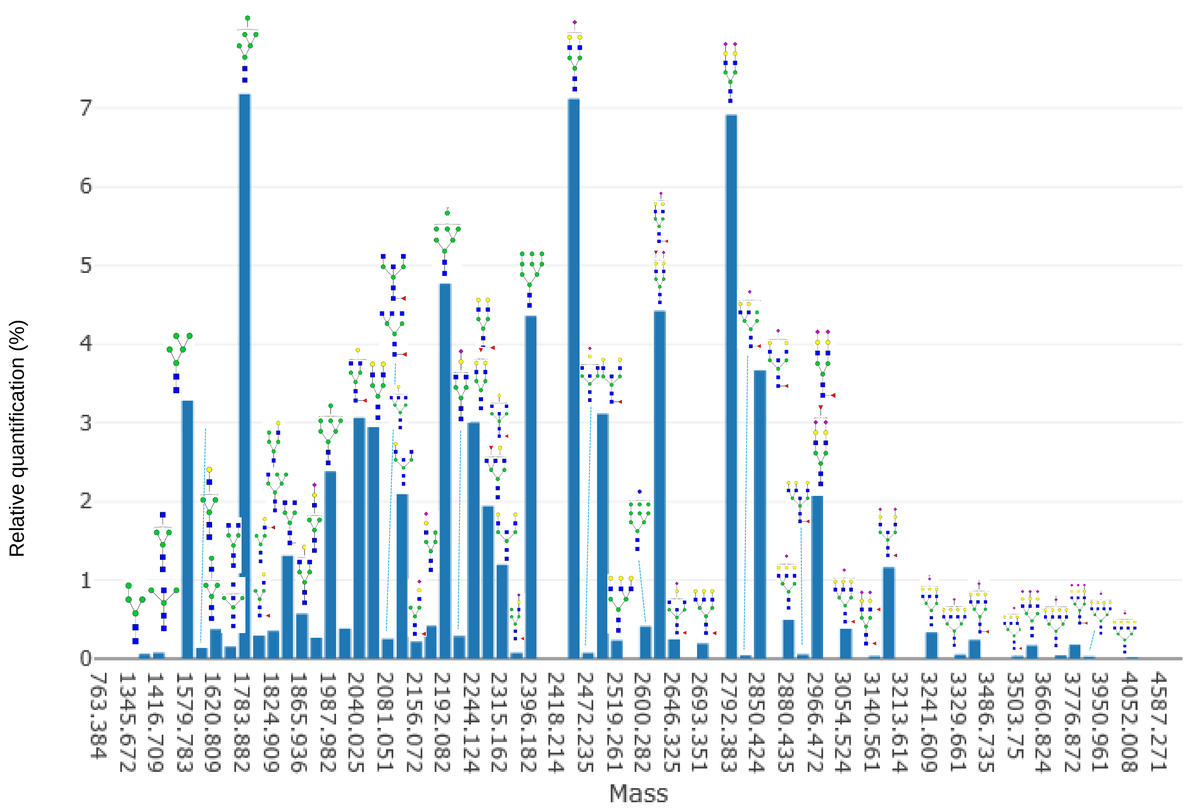

Fig 6. Relative abundance of N-glycans present in serum

Conlusions

- In alpha- fetoprotein (AFP), 16 N-glycan structures were identified the degree of fucosylation was found to be 18.6%, while sialylated and branched glycans were 5.5% and 3.4%, respectively. The predominant glycoform was A2G2 (H5N4 62.8%), followed by FA2G1 (H4N4F1 7.4%) and FA2G2 (H5N4F1 4.5%).
- The major N-glycans groups in AFP were bi-antennary complex structures with high levels of galactosylation, fucosylation, sialylation while hybrid structures are found in lower levels. Our data indicated that the glycosylation pattern of AFP was consistent with the literature and the masses of the main glycans were as expected.
- 52 N-glycan structures were identified in serum samples consists mainly of neutral and sialylated glycans. The major groups were bi-antennary, bisected, tri- and tetra-antennary N-glycans, with different levels of sialylation.
- Employing permethylation strategy we improved the ionization and detectability of sialylated glycans. Using our workflow of glycan analysis even minor glycans such as tri- and tetra-antennary N-glycans were detected in serum.
- We have presented a rapid, accurate, sensitive and reproducible N-glycan analysis from commercially available standard (AFP) and complex sample (serum) for identification and relative quantification by MALDI-TOF-MS.
- We believe that this method can be used for identification of N-glycans of complex samples and glycoproteins for biomarker discovery in colorectal cancer.

References

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[4] https://www.ludger.com/presentation/glycan-permethylation/

Acknowledgements

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