GRAPHITIZED CARBON COLUMNS FOR THE ANALYSIS OF PROTEIN GLYCOSYLATION IN CAPILLARY LC

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Introduction

Protein glycosylation is involved in important biological functions such as cell differentiation, immunogenisis, protein solubility, cell recognition, cell adhesion and signal trafficking. Protein glycosylation is one of the most important but also the most complex of the post-translational modifications.

The analysis of protein glycosylation is particularly difficult because the range of carbohydrate units can be connected in a number of ways leading to a large number of possible isomers. Graphitised carbon packing material shows a unique ability the resolve the isomeric forms of the glycan structures. In collaboration with Proteome Systems we have developed a platform for the analysis of protein glycosylation. The approach includes all steps from the sample preparation, the separation of the oligosaccharides using the carbon packed capillary columns and MS/MS detection and the bio-informatics data analysis.

In a typical experiment a prepared sample would be separated by gel electrophoresis, the protein bands associated with glycosylated proteins would be isolated and the carbohydrate structures are enzymatically cleaved off the protein. These structures are then separated on a graphitised carbon capillary LC column and analysed using MS/MS. The resulting mass patterns are referenced against a glycol database.

Protein Glycosylation

Protein glycosylation is the most common post-translational modification in proteins. There is a variety of carbohydrate units that can be incorporated through any of its hydroxyl groups which leads to a large number of possible isomers and a very complex system to analyse.

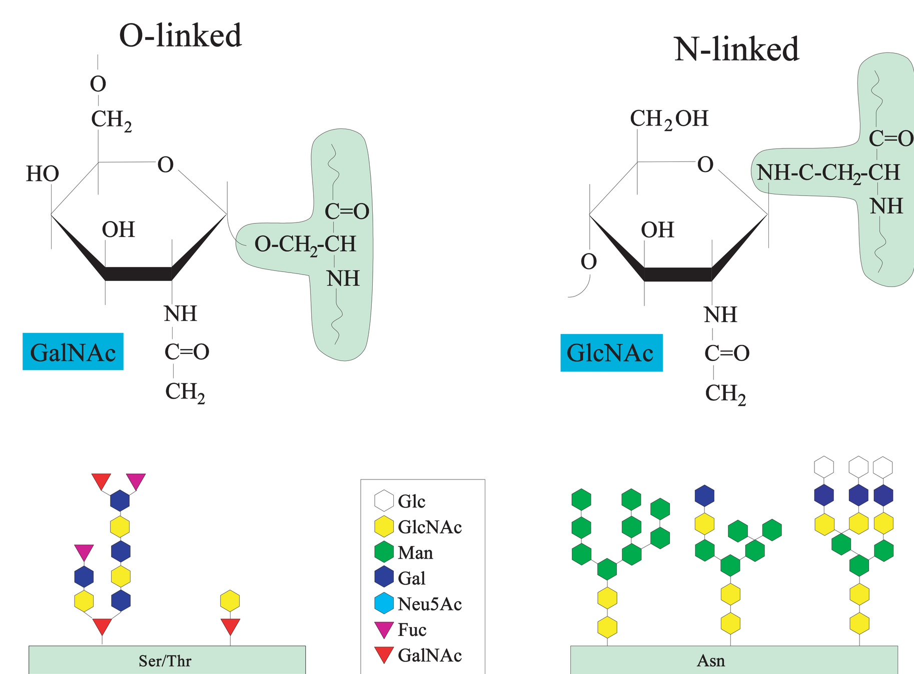


Figure 1: Possible carbohydrate structures on the protein backbone

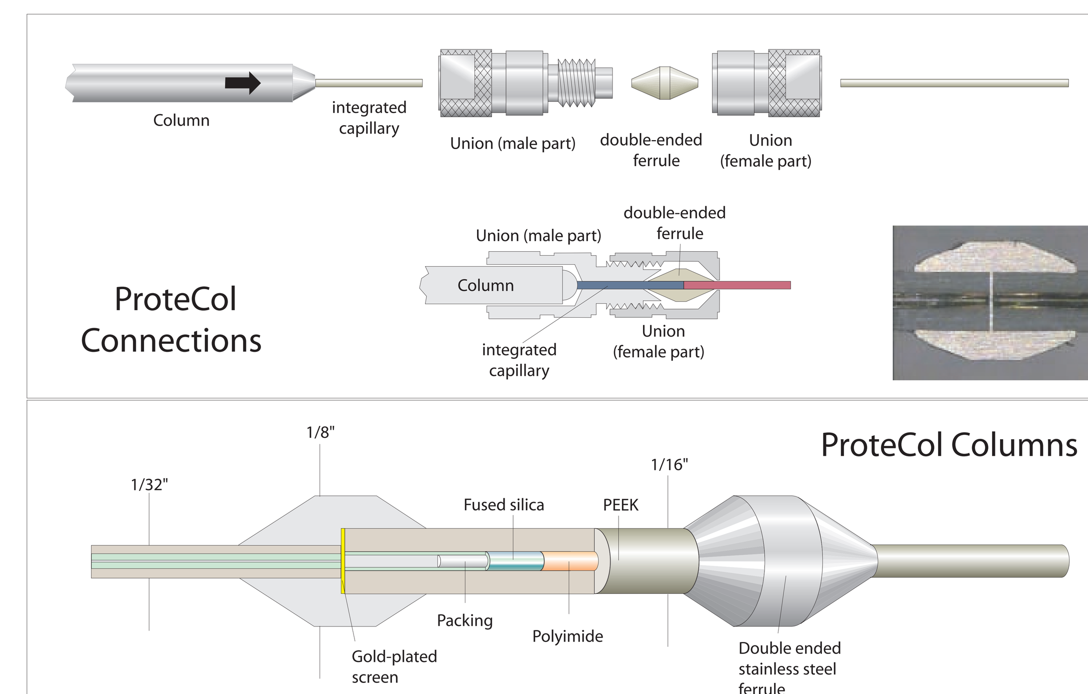
NUMBER OF POSSIBLE ISOMERIC PEPTIDES AND OLIGOSACCHARIDES (PYRANOSE RING ONLY)

Composition	Product	Number of Peptide	Number of Oligosaccharides
X-X	dimer	1	11
X-X-X	trimer	1	176
X-Y-Z	trimer	6	1056

Design Features Of The ProteCol™ Range

The ProteCol™ range of columns and connection is designed to minimize void volumes and non-specific interactions - the two main sources of decreased sensitivity in capillary and nano-LC.

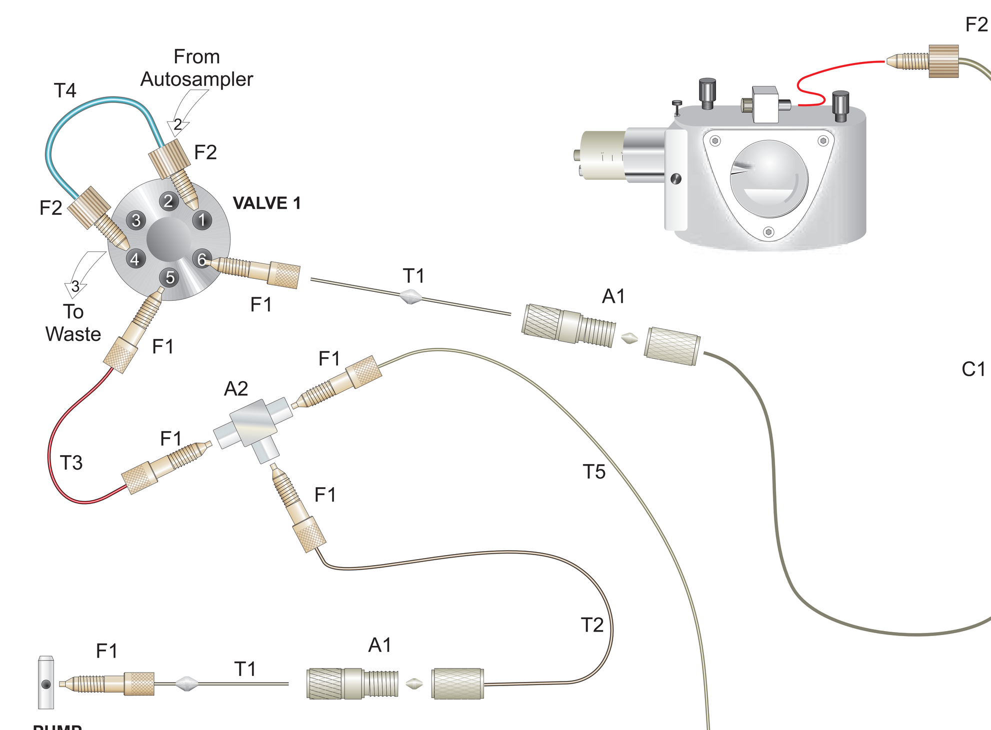
* all capillaries (column body, connection tubing and in-line filters) are made of deactivated PEEKsil tubing (deactivated fused silica coated with PEEK)
* all screens and filters are made from gold-plated stainless steel and have a 150μm thickness to minimize band spreading.
* columns have integrated connection tubing
* all capillary ends are pecision square cut to allow zero-volume butt connections



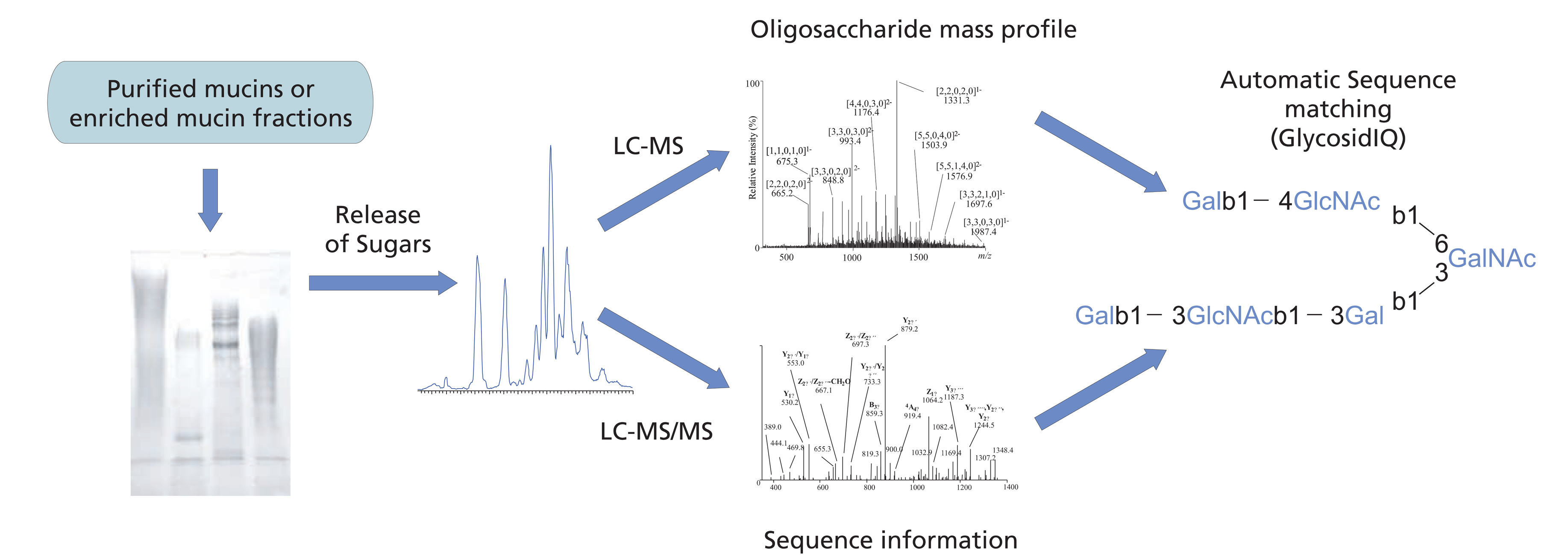
Oligosaccharide Analysis Made Easy In Kit-form

In connection with Proteome Systems we assembled a protein glycosylation analysis kit which contains the columns, connection tubing, filters, accessories, a calibration standard and step-by-step instructions to make the analysis of protein glycosylation accessible and reproducible. The kit is incorporated into a larger platform including all neccessary steps from sample preparation to data handling and data analysis.

The figure below shows the LC setup for Carbohydrate analysis by LC-MS².



Approach for Analysis of Mucin Glycosylation



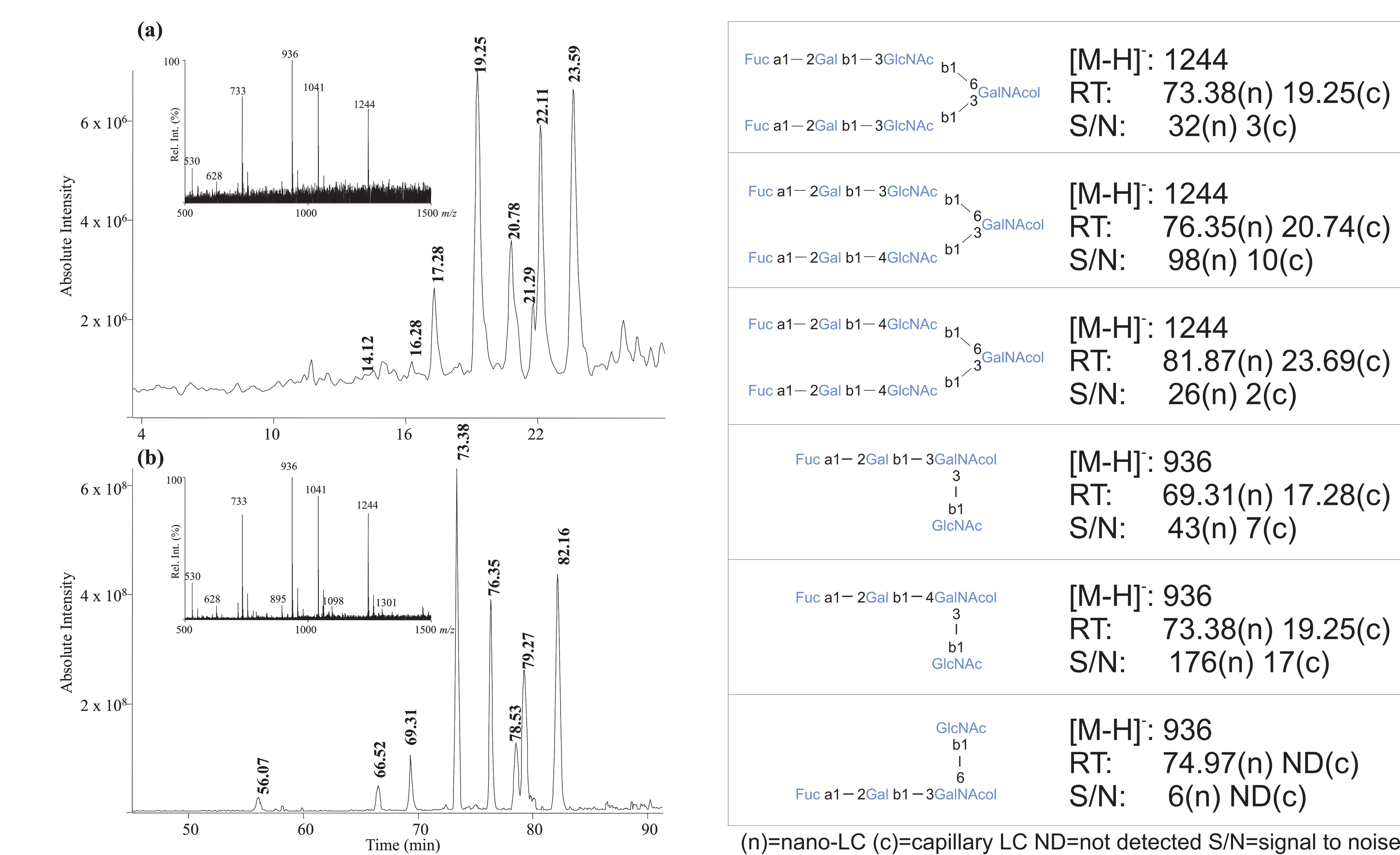
Detection of neutral O-linked oligosaccharides by negative ion capillary- and nano-LC/MS2

Neutral oligosaccharides released from small intestinal mucosal proteins from 6 rats were isolated and analyzed byt negative ion capillary LC-MS.
Oligosaccharide samples (10 μL) were dissolved in water and injected via a Surveyor autosampler and HPLC system (Thermo Finnigan, San Jose, CA) andanalyzed using a LCQ-XP+, Thermo Finnigan mass spectrometer.

Columns: ProteCol Hypercarb (5μm/250Å) 100 mm x 0.3 mm (capillary LC) or 100 mm x 0.15 mm (nano LC)
Solvent A: 10 mM ammonium bicarbonate
Solvent B: 10 mM ammonium bicarbonate in 80 % acetonitrile
Gradient: 0- 37.5% B over 27.5 min for capillary LC or 2-30%B over 90 minutes for nano-LC
Flow rate was 110-120 μL/min, split down before the autosampler to 6 μL/min (capillary LC) or 0.5 μL/min (nano LC).
ESI spray current 3.5kV (capillary LC) 1.1kV (nano-LC)

Some examples to highlight column selectivity for oligosaccharide isomers Approach for Analysis of Mucin Glycosylation

Some examples to highlight column selectivity for oligosaccharide isomers



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