Lebrilla Group Research

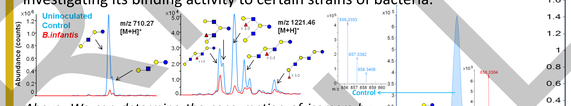
http://chemgroups.ucdavis.edu/~lebrilla/

Comprehensive "Omics"

Milk Glycomics

▲ Fuc ○ Gal ● Glc ■ GlcNAc ◆ Neu5Ac

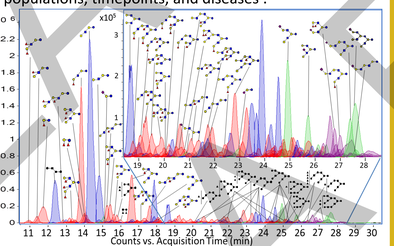
Oligosaccharides in human milk (HMOs) represent an abundant and diverse group of bioactive molecules. Although they do not provide direct nutritional value to the infant, they are beneficial to the growth and development of the infant's gut flora. The protective and prebiotic effects of HMOs are what drives studies today. Recently we have been investigating its binding activity to certain strains of bacteria.



Above: We can determine the consumption of isomers by specific strains on an isomer specific level using HPLC/Chip-TOF-MS. Shown here are the consumption profiles of bifido infantis overlaid against the uninoculated control.

Recent Publications: Hong et al. Anal. Chem. 2014; Guerrero et al. Int. J. Mass Spec. 2013; Peacock et al. Ag. Food and Chem. 2013; Totten et al. J Proteome Res. 2012

After creating a high throughput method, we can now quantify HMO profiles across a large batch of different populations, timepoints, and diseases .



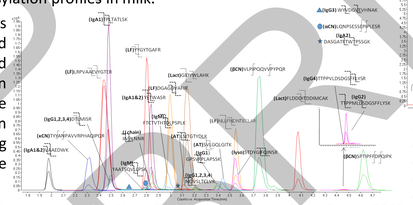
Above: An HMO pool sample was analyzed using HPLC Chip/TOF-MS to determine glycan signal. All known compounds were extracted, overlaid, and structurally identified/annotated using an in-house library.

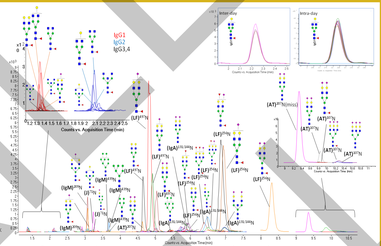
Milk Glycoproteomics

▲ Fuc ● Man ○ Gal ■ GlcNAc ◆ Neu5Ac

Proteins are also one of the major components in milk and a large subset of milk proteins are glycosylated. These glycoproteins make prominent contributions to the health benefits ascribed to consuming milk. Our research aims at establishing structure-specific functional benefits of the unique protein glycosylation profiles in milk.

To this end, strategies involving MS-based proteomics, gel-based quantitative protein profiling, and site specific glycosylation analysis are being used to establish the milk glycoproteome.



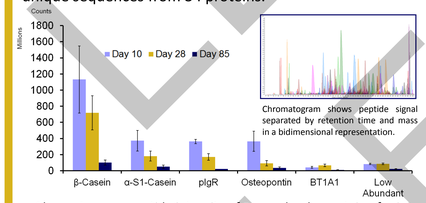


Left: Multiple reaction monitoring (MRM) transitions for 10 milk proteins and milk glycopeptides (above) analyzed and annotated by UPLC-QqQ-MS. Analysis is reproducible inter- and intra-day. Proteins included Beta-casein, kappa-casein, alpha S1 casein, alpha-lactalbumin, lactoferrin, secretory IgA, IgG, IgM, alpha 1-antitrypsin and lysozyme.

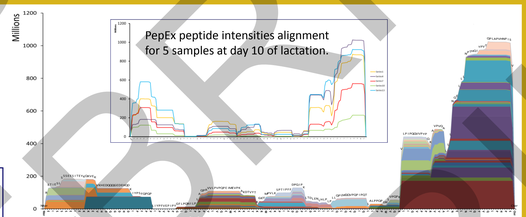
Recent publications: Smilowitz et al. J. Nutr. 2013 , Nwosu et al. Anal. Chem. 2013.

Milk Peptidomics

Milk is a self-digesting biofluid rich in proteolytic enzymes. A comprehensive study of the peptide content in human milk has revealed a vast diversity of naturally occurring peptides (NOPs). A human milk NOPs library has been created with more than 800 unique sequences from 34 proteins.



Above: Average peptide intensity of 5 samples by protein of origin and moment of lactation.



Above: ß-casein NOP intensities alignment using PepEx. Each color band represents the intensity contribution of an NOP. Although the whole protein is digested to some extent, certain regions of ß-casein are more digested than others. The NOPs coming from different protein areas are related with different biofunctionalities.

Human milk samples from 5 different mothers at three different timepoints of lactation were studied via HPLC Chip/Q-TOF. NOPs intensities were grouped, mapped and interpreted using an in-house software. *Recent Publications: Dallas et al. J. Proteome Res. 2013, Guerrero et al. Mol. Cell. Proteomics. 2014*

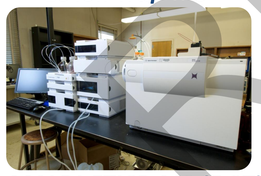
Instruments

HPLC Chip/TOF-MS

Microfluidic PGC Chip

HPLC Chip/Q-TOF-MS

LC Ion Trap-MS



UPLC-ESI-QqQ-MS

MALDI-FTICR-MS (7.0T) IRMPD/CID

NanoESI-FTICR-MS (9.4T) IRMPD

Rapid-Throughput

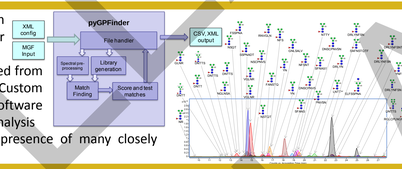
GX-274 Liquid Handler/SPE

96-well plate SPE

Above, left: Automated Solid Phase Extraction (SPE) via robotic liquid handler. (Above, right) 96 well plate via centrifugation allows us to purify a total of 400 samples from beginning to end within 2 hours.

Software

In-house software platforms are in constant development. pyGPFinder assists in the discovery and annotation of glycopeptides derived from a variety of proteolytic digests. Custom chromatographic alignment software allows us to run high throughput analysis on oligosaccharides even in the presence of many closely eluting isomers.



Clinical Glycomics/Biomarkers

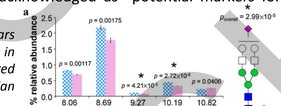
Cancer Biomarkers

▲ Fuc ● Man ○ Gal ■ GlcNAc ◆ Neu5Ac

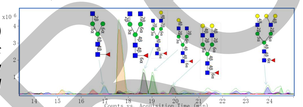
No.	Name	RT (min)	Mass(Da)	Relative Abundance (%)	Structure
1	N5402a	24.9	2224.80	100.000	
2	N5402b	17.9	2224.81	33.925	
3	N3410a	17.7	1464.56	25.585	
4	N4410a	18.5	1826.62	25.094	
5	N5401a	21.3	1933.71	23.474	
6	N5401b	14.5	1933.71	18.703	
7	N5411a	23.2	2079.77	17.994	
8	N5412a	26.8	2370.85	15.182	
9	N5411b	15.8	2079.77	13.033	
10	N5401c	20.8	1933.71	10.819	

Glycosylation is an important determinant of protein function and has been shown to mediate many cancer-related processes and has recently been acknowledged as potential markers for diseases.

*Right: Average relative abundances and standard error bars associated with five structural isomers of $Hex_5HexNAc_4FucNeuAc$ in ovarian cancer cases (striped pink, n=48) and controls (checkered blue, n = 46). *Statistically significant difference between ovarian cancer cases and controls.*



We have recently optimized a rapid-throughput method for comprehensive, isomer-specific chromatographic profiling of native serum glycans and applied it to glycan biomarker discovery. In the near future we will apply a rapidly developing structural serum N-glycan library featuring isomers with identifiers, relative abundances, retention times, accurate masses, and tandem MS spectra (*partial library shown on left*).



Additionally, we are characterizing monoclonal antibody (mAb) glycan profiles. Glycosylation may affect the therapeutic efficacy, pharmacokinetics, immunogenicity, folding and stability of these proteins. Shown (right) is an annotated extracted compound chromatogram of Eculizumab.

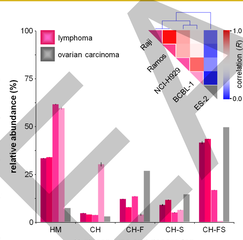
Recent Publications: Kim et al. Cancer Epid. Biom. Prev. 2014; Ozcan et al. Cancer Prev. Res. 2014. ; Hua et al. Anal. Chem. 2013

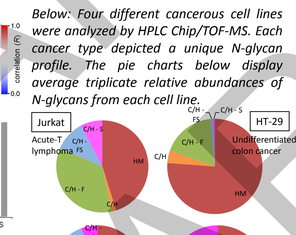
Cell Surface Glycosylation

Glycosylation on the cell surface membrane plays an important role in cell signaling and bioactivity. We have optimized a method to isolate and enrich for cell membrane glycans and use analytical tools to study them. In recent studies, we profiled cell membrane N-glycans from a variety of cell lines, and found that the same types displayed similar trends.

Graphed to the right is a color-coded representation of the Pearson correlation coefficient (*R*) between each pair of cell lines (Red- high correlation, blue- low correlation). Relative abundances are shown for each glycan class. ES-2 is clearly distinct from the group of lymphoma cell lines.

Recent Publication: Hua et al. J. Proteome Res. 2013; An et al. Mol. Cell. Proteomics 2013.





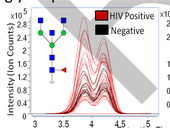
Above: Glycan class profiles of four B-cell lymphoma cell lines: Raji, Ramos, NCI-H929, BCBL-1 and one ovarian carcinoma cell line (ES-2) analyzed by HPLC Chip/TOF-MS.

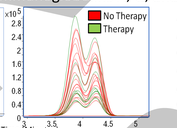
Below: Four different cancerous cell lines were analyzed by HPLC Chip/TOF-MS. Each cancer type depicted a unique N-glycan profile. The pie charts below display average triplicate relative abundances of N-glycans from each cell line.

Markers for Autoimmunity

▲ Fuc ● Man ○ Gal ■ GlcNAc ◆ Neu5Ac

Over the last decades, several successful studies have emerged on the detection of candidate glycan biomarkers for several diseases including auto-immune diseases. Most studies have focused on whole biofluids, and changes in specific glycoforms of proteins may result in more important biomarkers, with improved sensitivity and specificity. Here, we present methods to investigate protein- and site- specific glycan profiles of the immunoglobulins A, G, and M in a quantitative manner.





Above: Overlaid ECCs of $Hex_3HexNAc_5Fuc_1$ found in HIV positive and controls (left) and therapy and no therapy groups (right)

Recent Publication: Hong et al. Anal. Chem. 2013; Ruhaak et al. Proteomics Clin. Appl. 2013

Below: Simultaneous absolute immunoglobulin quantitation and glycosylation analysis directly from serum allows us to apply this method to clinical studies.