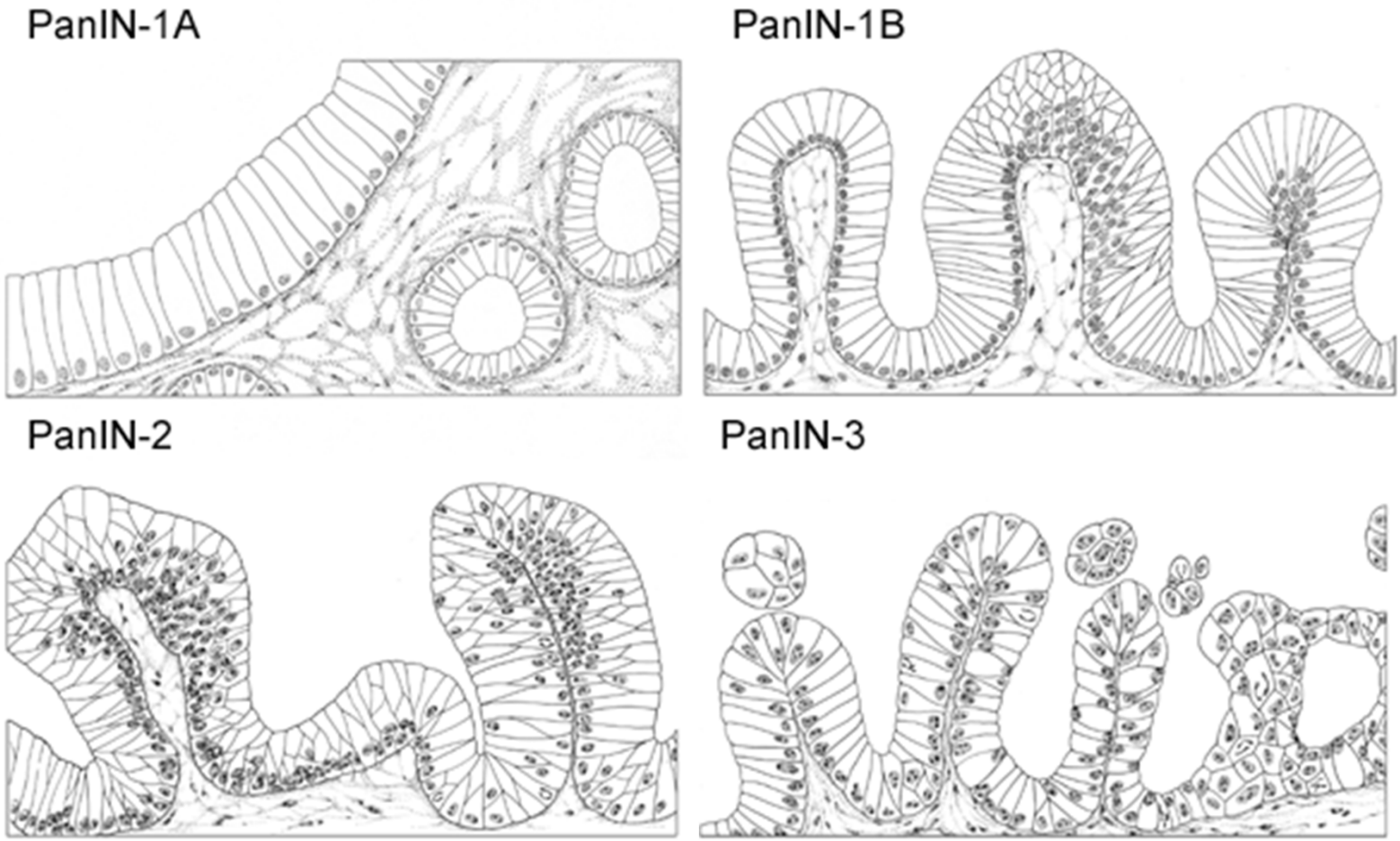
Investigators and Scientific Areas

- Discovery of Cancer Associated Glycans
  - Michael Pierce, Lance Wells, University of Georgia
  - Bill Hancock, Northeastern University
  - Milos Novotny, Indiana University
- Antibodies to Tumor-Associated Glycans and Glycopeptideepitopes
  - Margaret Huflejt, New York University.
  - Denong Wang, Ten Feizi, Stanford University, Imperial College London
  - Tony Hollingsworth, Eppley Institute, University of Nebraska Medical Center, Henrik Clausen and Ola Blixt, University of Copenhagen
  - AjitVarki, Richard Schwab, University of California San Diego

ALLIANCE of GLYCOBIOLOGISTS
For Detection of Cancer and Cancer Risk

The Problem – Diagnosis of Early Cancer and Determination of Cancer Progression



Glycomics Technologies applied to Cancer

- Glycotranscriptome analysis
- Glycan and glycoprotein analysis of tissues, serum, fluids
- Glycan and glycopeptide arrays for detecting unique autoantibodies

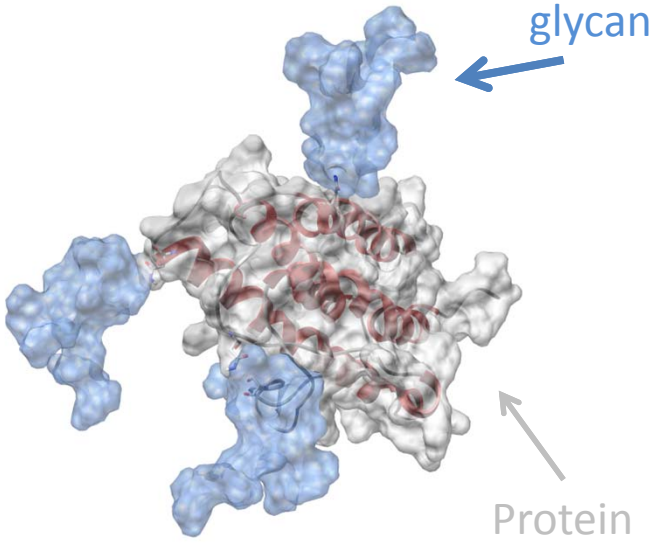
Collaborations

- Clinical samples and technologies from several institutions
- Unique resources
  - Pancreatic cancer rapid autopsy samples (Nebraska)
  - Prostate cancer samples (Stanford)
  - Discovery and Reference sets of sera and plasma from EDRN
- Collaborations with Investigators from EDRN, SPORE program, and P01s

Targeted Glycoproteomics: exploiting glycan expression on specific glycoproteins to identify potential cancer biomarkers

Tumor Glycomics Laboratory, Univ. of Georgia, and UGA Cancer Center

- **Identify glycan** changes by comparison of direct glycan analyses, glycotranscriptome qRT-PCR analyses, or lectin/antibody binding to cancer and control tissues. **Target** the glycoproteins that express these glycans (Methods developed in NCRR Glycomics Center, UGA)



Space-filling model of a secreted glycoprotein. Its three glycans are depicted in blue; polypeptide in white/red. Courtesy of R. Woods, CCRC, UGA

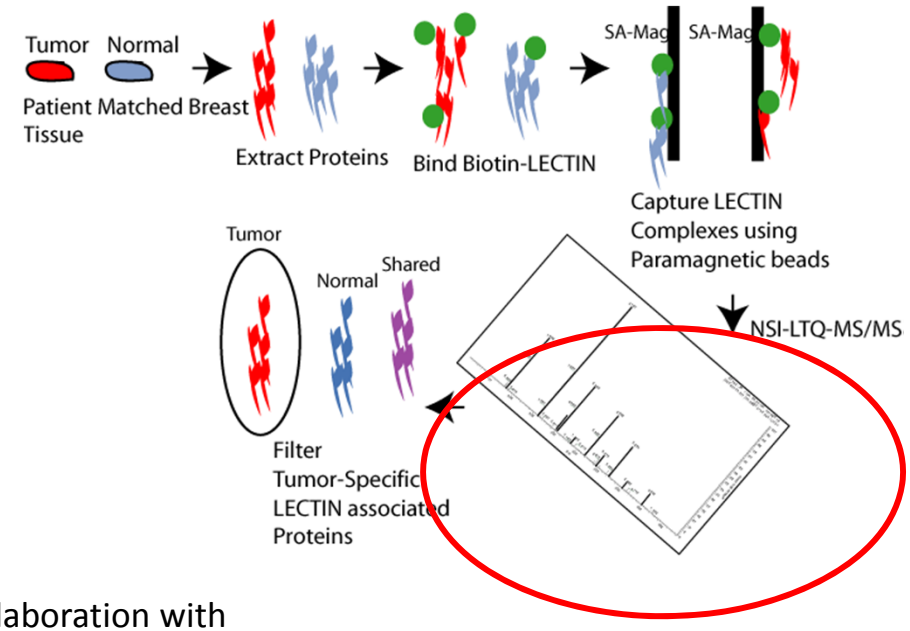
- Use lectins/antibodies to **separate** glycoproteins with these glycan changes
- **Identify** cancer-specific glycoproteins expressing Targeted Glycans via proteomics
- **Verify** and **validate** in tissue and serum with a two-step lectin:antibody assay
- Markers require 2-dimensional specificity: Protein **plus** Glycan expression

Application of targeted glycoproteomics methodology to discovery of invasive ductal breast carcinoma markers

•The lectin L-PHA binds glycans not present in breast epithelia, but which are expressed in late adenoma and carcinoma.

• Utilized Lectin L-PHA to target glycoproteins that expressed the glycan of interest in four cases of breast carcinoma with matched, non-diseased tissue controls.

• Identified 12 glycoproteins bound by L-PHA common to all four carcinomas but not in any of the controls.



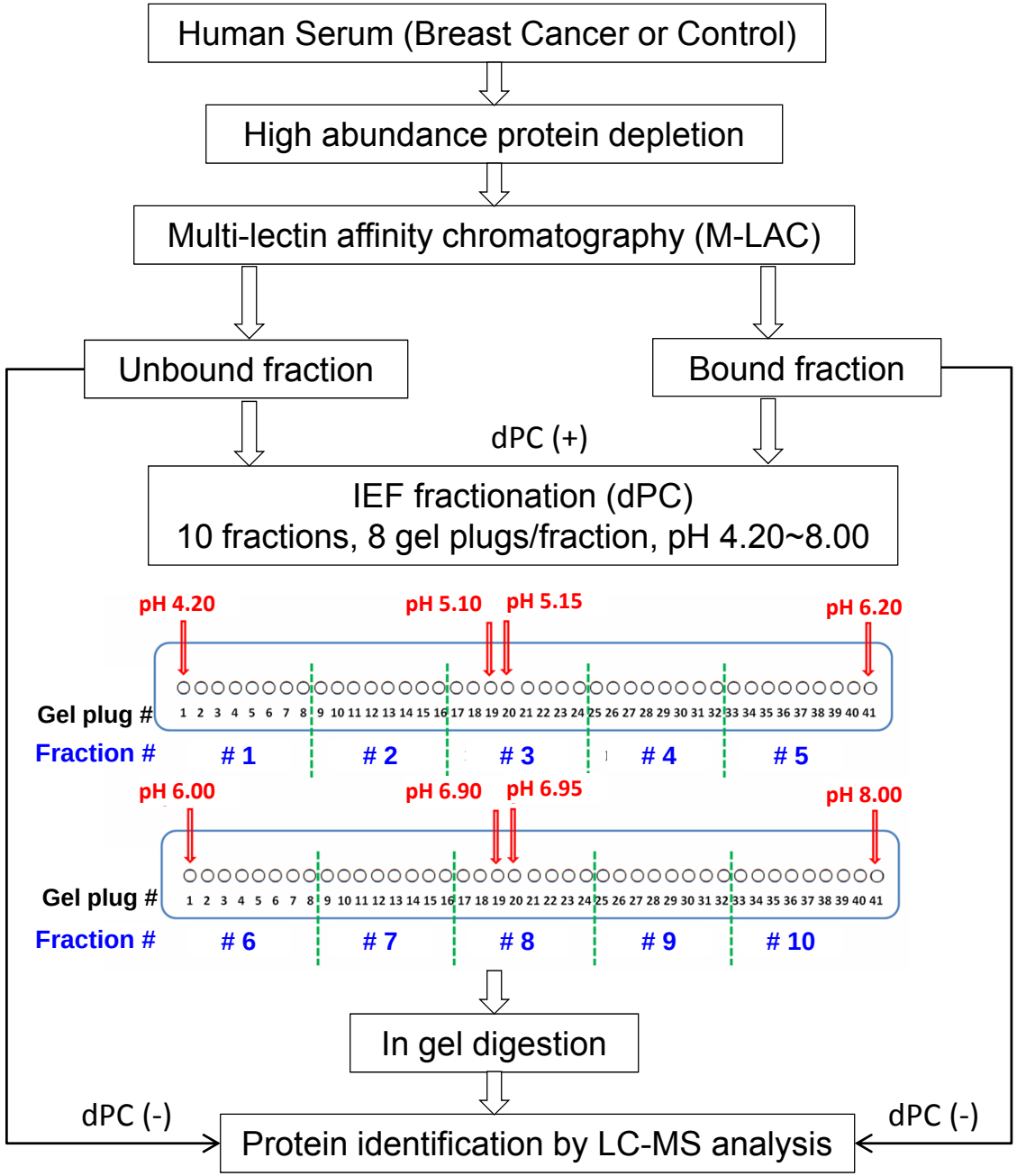
Two glycoproteins have been verified in breast carcinoma tissue and serum.

PERIOSTIN and **MIMICAN** are found **bound by L-PHA only** in cancer tissues and serum

Both are EMT-expressed, secreted glycoproteins. They are now being validated as potential serum markers.

Collaboration with
Dr. Ruth O'Reagan
Emory Winship
Cancer Center

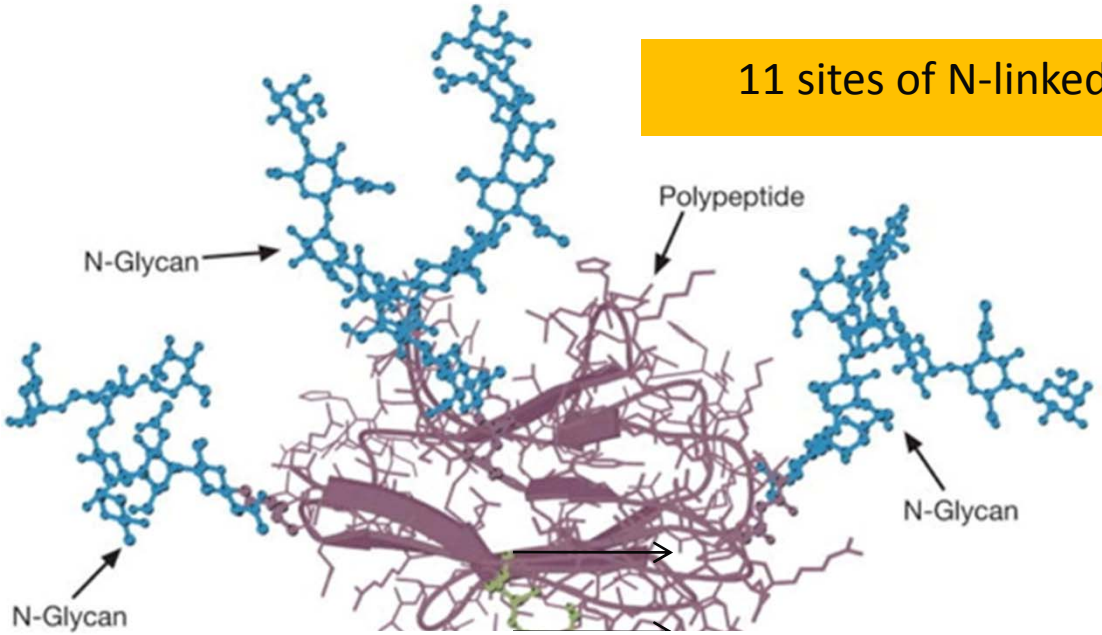
Abbott KL *et al.* Targeted glycoproteomic identification of biomarkers for human breast carcinoma. *J. Proteome Res*. 2008 Apr;7(4):1470-80.



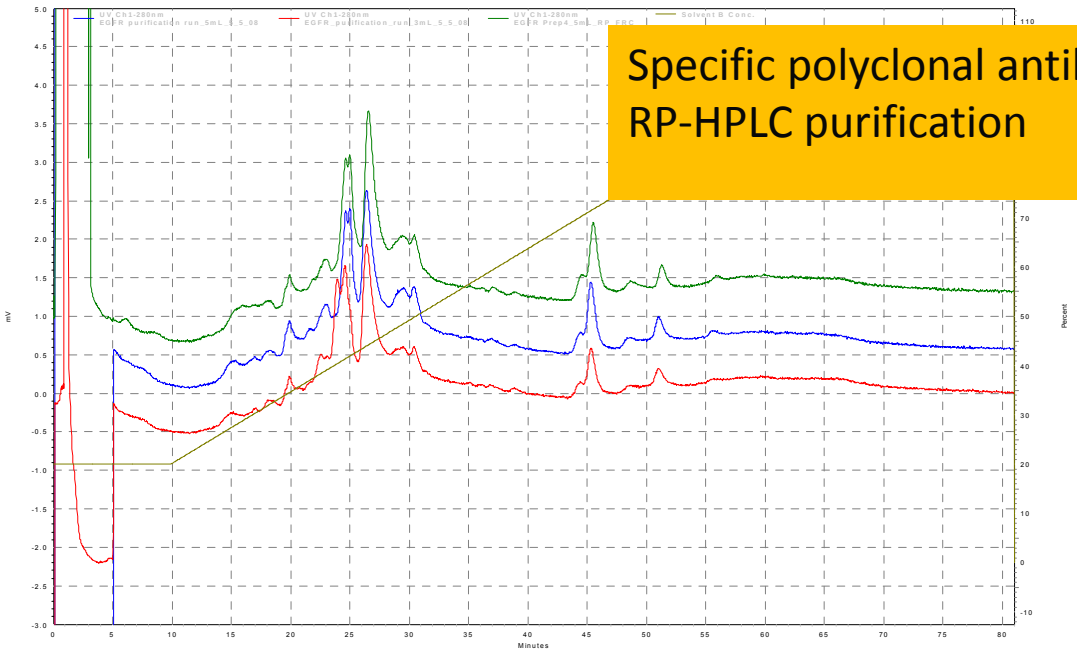
Platform used for the analysis of the breast cancer and control sera.
Four separation approaches –
•Depletion,
•Glycoprotein fractionation
• IEF fractionation using dPC and
•RP-LC/MS peptide separation.

Periostinwas identified with high confidence.
The isoelectric focusing profile showed a shift to more acidic pI values in the disease samples, which indicates a greater sialic acid content in breast cancer.

Targeted Approach EGFR (A431 cells)



11 sites of N-linked glycosylation



Specific polyclonal antibody pull down
RP-HPLC purification

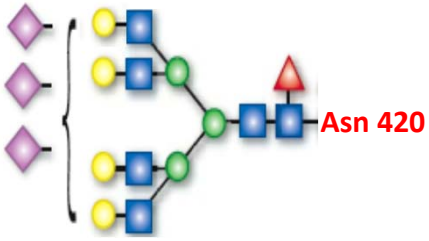
Q. Lu

Glycan analysis by LC with LTQ-FTMS of EGFR glycopeptide eluted at 25 to 25.5 minutes from a reversed phase column

CID → m/z 1169.73 (5+)

QHGQFSLAVVSLNITSLGLRSLK (Asn 420)

Tetra-antennary with 3 sialic acids

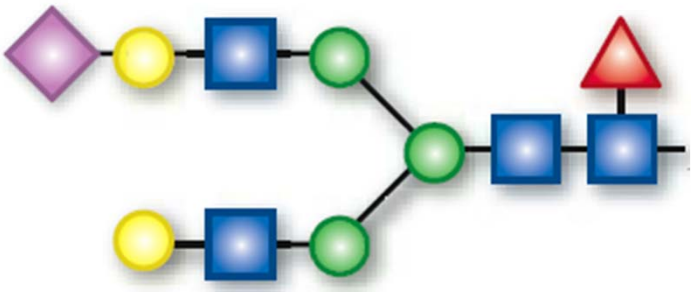


Q. Lu, Billy Wu

EGFR Glycosylation (A431 cell line)

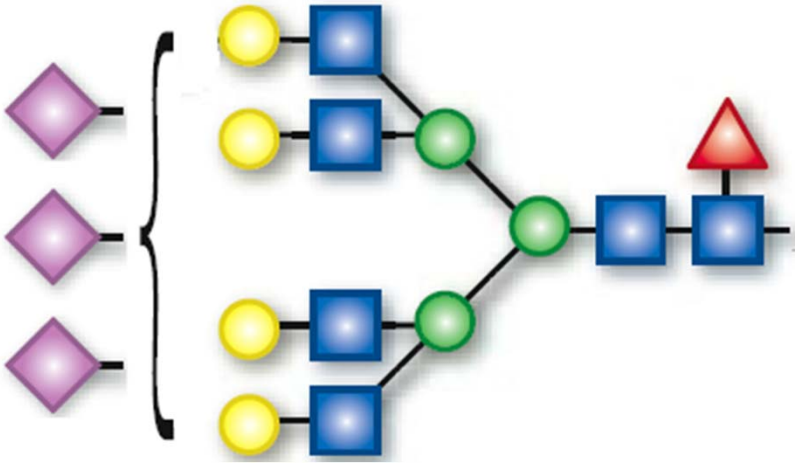
Major glycoform at Asn 420 site

Membrane bound

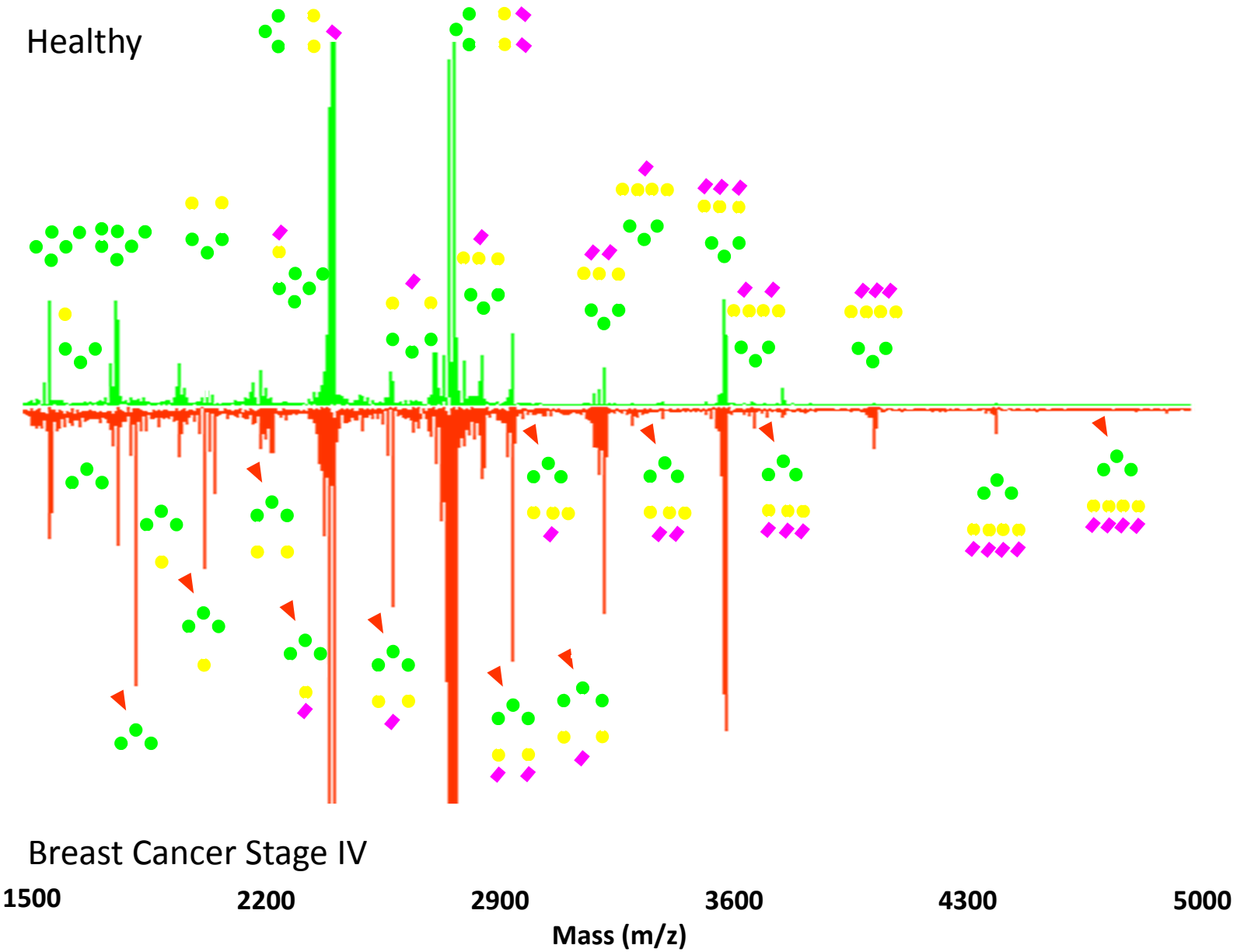


bi-antennary structure with
1 terminal sialic acid

Soluble form (secreted)

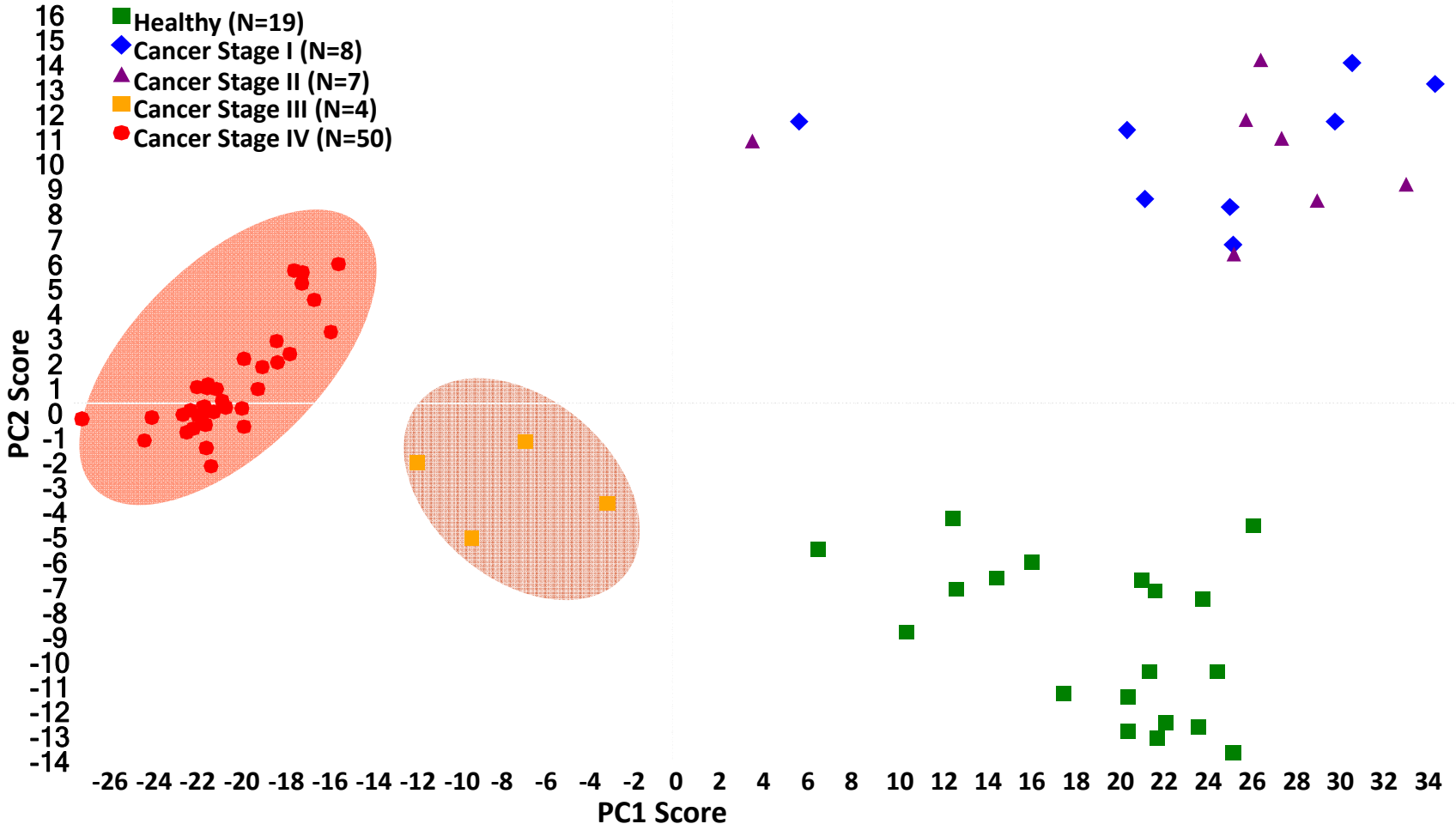


tetra-antennary branches with
3 terminal sialic acids



Z. Kyselova, Y. Mechref, P. Kang, J. A. Goetz, L. E. Dobrolecki, G. Sledge, L. Schnaper, R. J. Hickey, L. H. Malkas, M. V. Novotny "Breast Cancer Diagnosis/Prognosis through Quantitative Measurements of Serum Glycan Profiles" ***Clin. Chem.***, in press.

PCA of MALDI/MS Profiling of Glycans Derived from Sera of Healthy Individuals and Breast Cancer Patients



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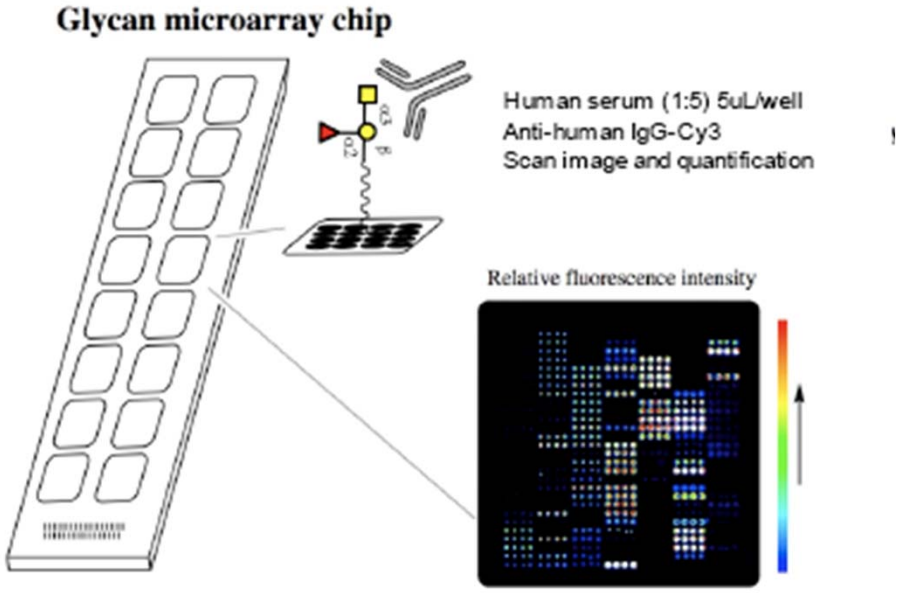
Microarrays for detecting antibodies with unique oligosaccharide and glycopeptide specificities



- Huflejt - ~ 300 unique oligosaccharides on microarrays
- Wang – Clustered oligosaccharides representative of prostate cancer
- Feizi – Arrays of oligosaccharides from mucins of ovarian cystadenomas
- Varki - Neu5Gc (*N*-Glycolylneuraminic acid)
- Clausen, Blixt, Hollingsworth – Glycopeptides containing specific tumor associated oligosaccharides (Tn, T, sialylTn, sialylT) attached to mucin peptides

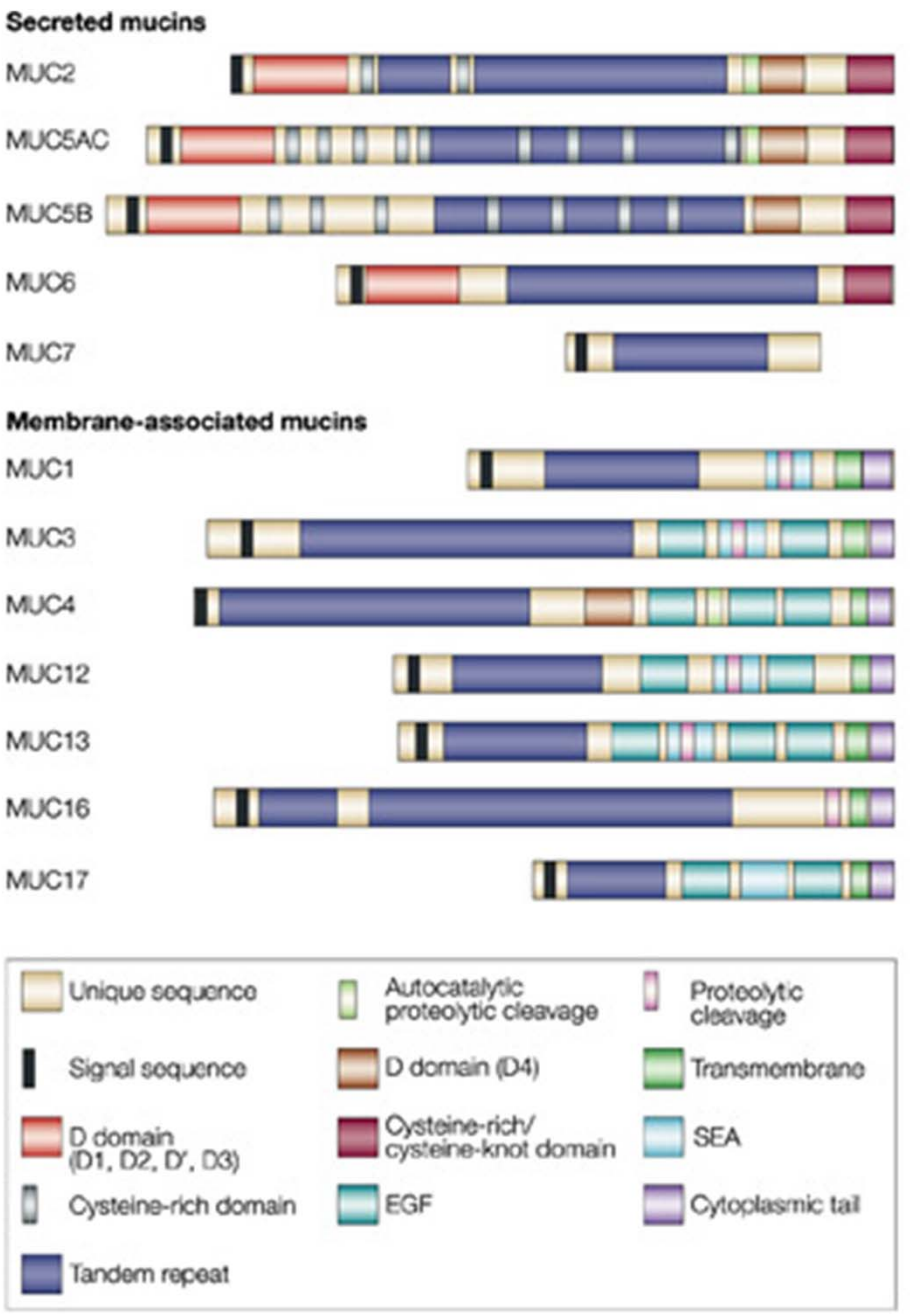
Mucins

21 genes

Common features:
Secretion into mucus layer
High MW glycoprotein
TANDEM REPEAT
Heavily glycosylated

Different tissues/ organs express differing sets of mucins

Tumors express different core proteins and differential glycosylation of these



Hollingsworth and Swanson, 2004 Nature Reviews | Cancer

The Glycopeptide array

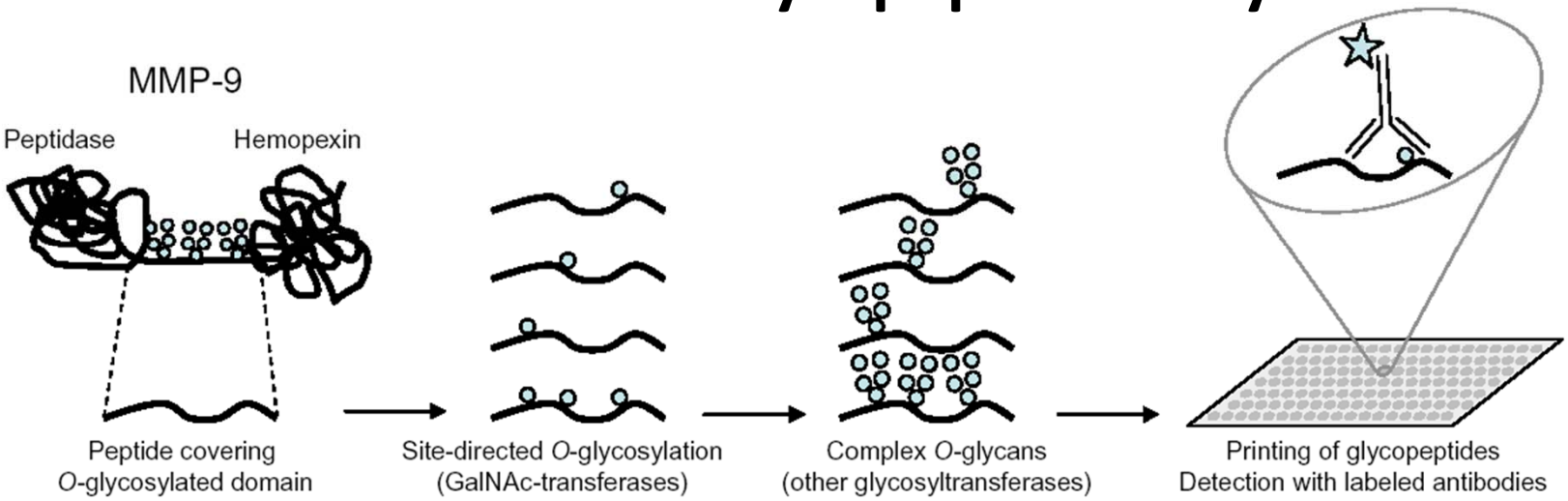
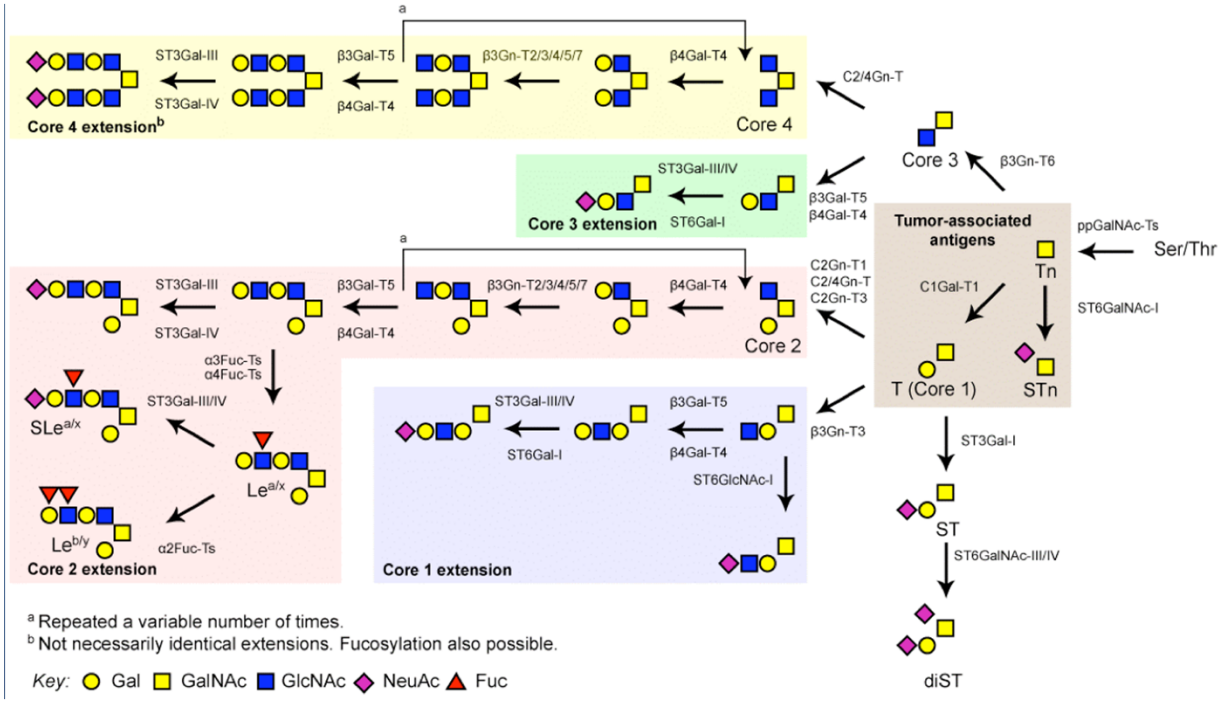


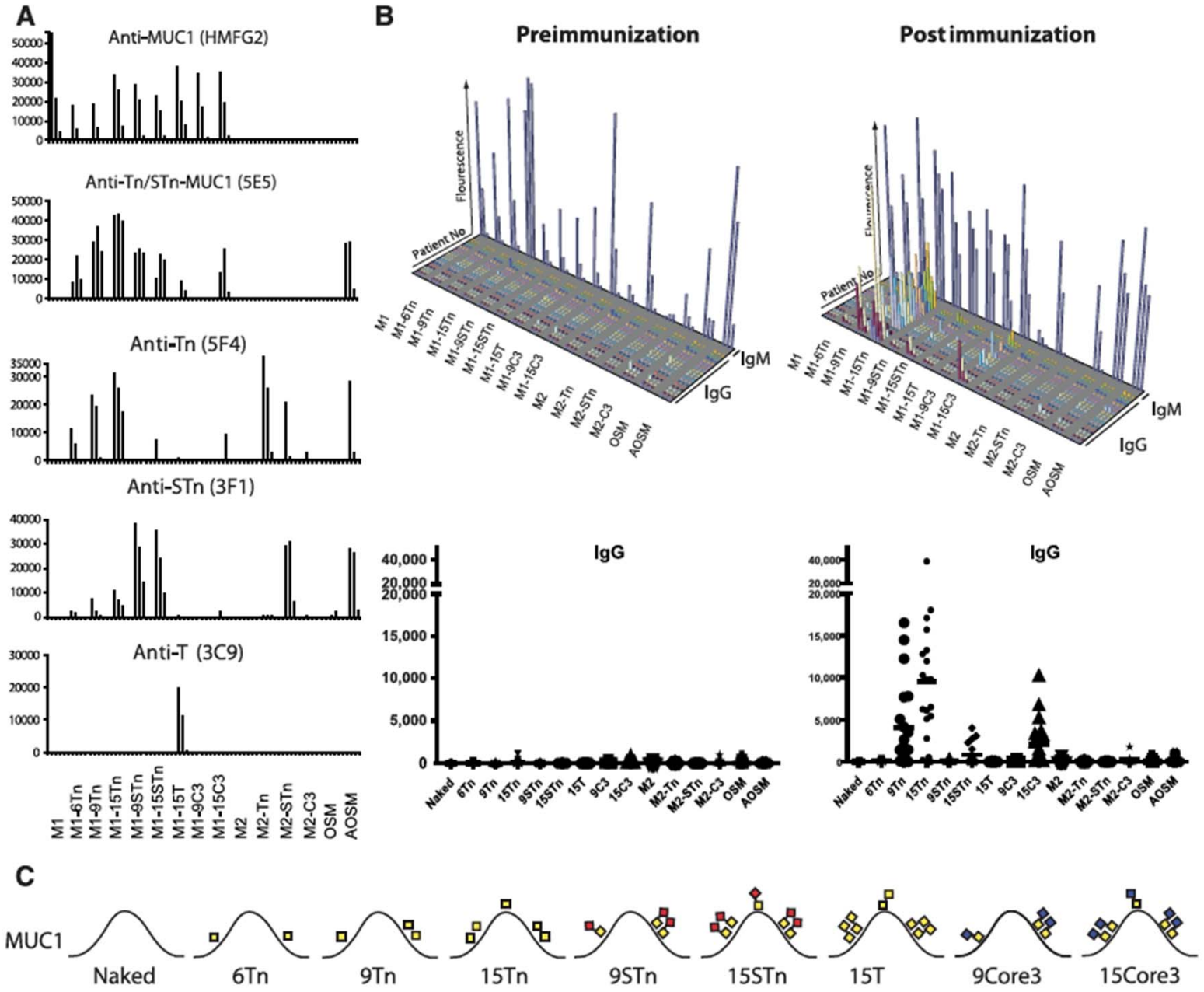
Figure courtesy M. Tarp, E. Bennett and H. Clausen

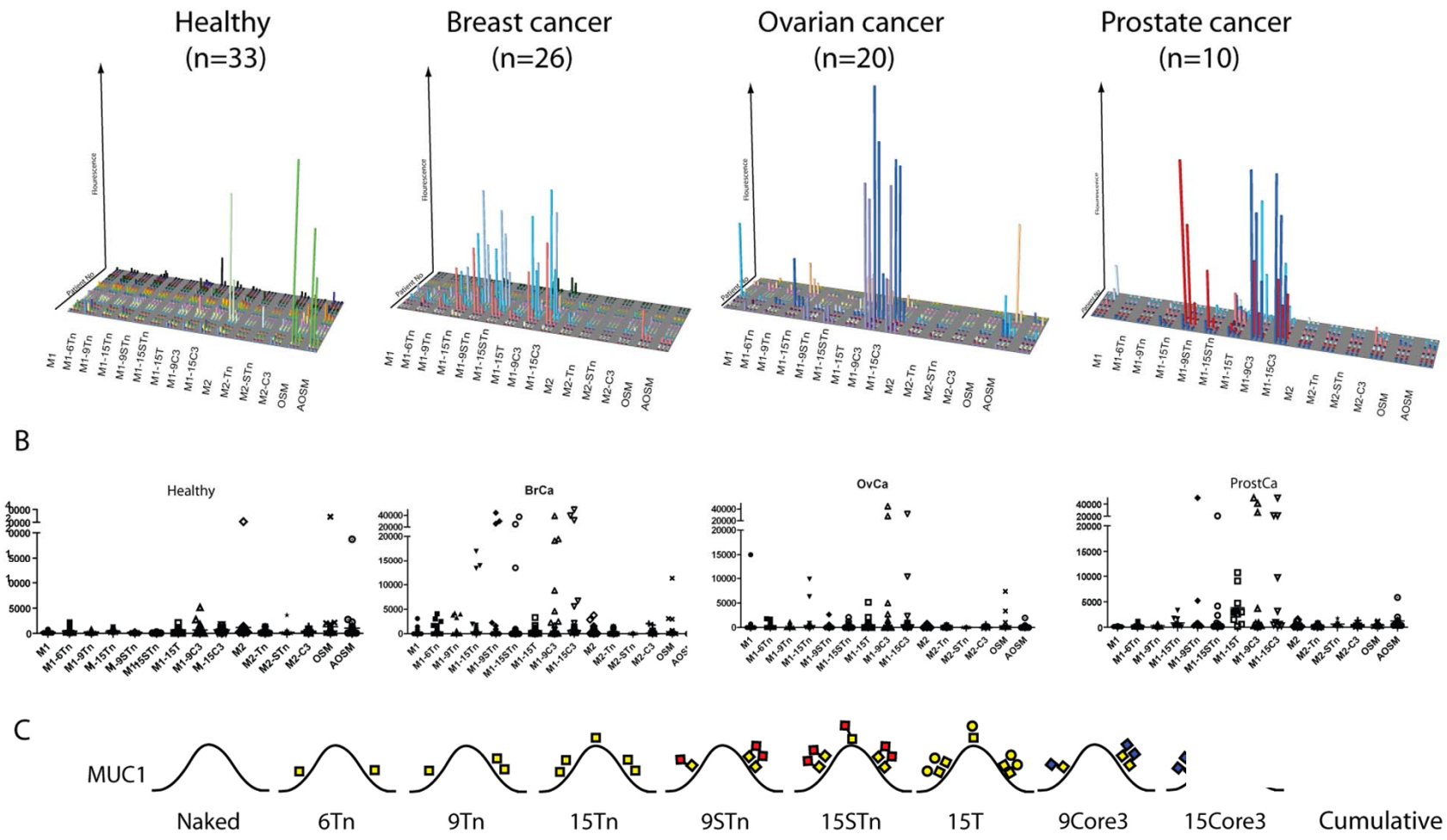


1st prototype

- **MUC-1 (60mer)**
- **6Tn-MUC-1 (60mer)**
- **9Tn-MUC-1 (60mer)**
- **15Tn-MUC-1 (60mer)**
- **9STn-MUC-1 (60mer)**
- **15STn-MUC-1 (60mer)**
- **15T-MUC-1 (60mer)**
- **9Core3-MUC-1 (60mer)**
- **15Core3-MUC-1 (60mer)**
- **MUC-2 (33mer)**
- **6Tn-MUC-2 (33mer)**
- **12Tn-MUC-2 (33mer)**

Immunization of cancer patients with STn on MUC1 yields glycopeptide specific antibodies





Core3-, STn- and Tn-MUC1 auto-antibodies are present in cancer patients with breast, ovarian, and prostate cancers.

Summary

- Application of state of the art technologies to define unique glycan structures associated with cancer progression
- Definition of antibody responses to unique oligosaccharide and glycopeptideepitopes during cancer progression
- Investigators interface with EDRN, SPORE program, PO1s, CFG