Mucinomics: understanding the complex relationship between mucins and human disease

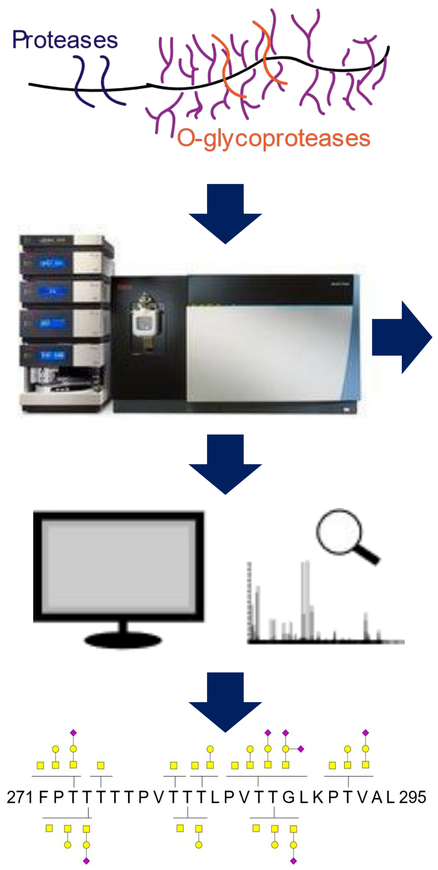
Valentina Rangel-Angarita[1], Deniz Ince[1], Keira Mahoney[1], and **Stacy A. Malaker**[1]

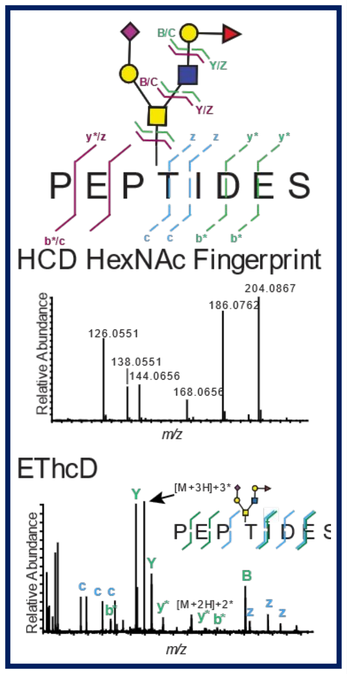
[1]Department of Chemistry, Yale University, New Haven CT

Yale

MASS SPECTROMETRY

Using a combination of **proteases and mucinases,** proteins are digested, fragmented, and resulting spectra are processed.

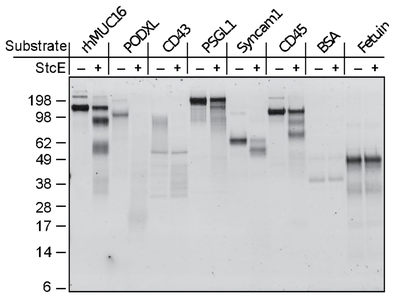




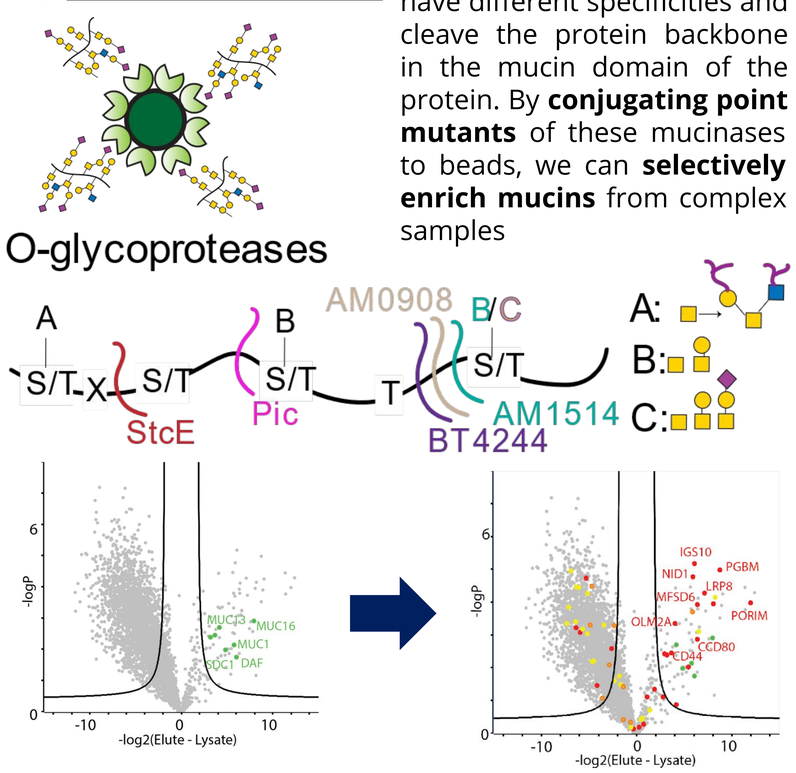
Electron and collisional dissociation methods are required to accurately assign glycosites.

Low charge density in mucins is compounded by their **low ionizability**, generating substantial challenges in analysis.

ENZYMATIC TOOLKIT

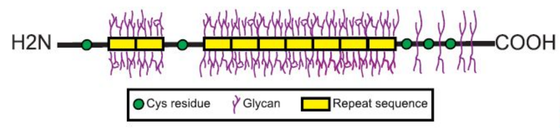


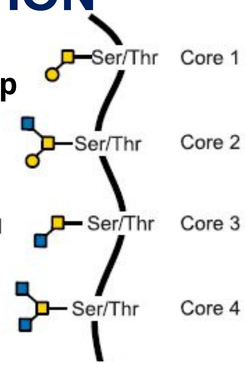
We have discovered and characterized mucin-selective O-glycoproteases, also termed **mucinases.** These mucinases have different specificities and cleave the protein backbone in the mucin domain of the protein. By **conjugating point mutants** of these mucinases to beads, we can **selectively enrich mucins** from complex samples



O-GLYCOSYLATION

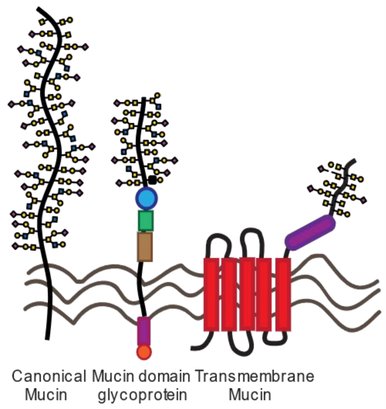
O-glycosidic bonds link glycans to an amino acid that contains **hydroxyl group** in the side chain, which is often Ser and Thr.





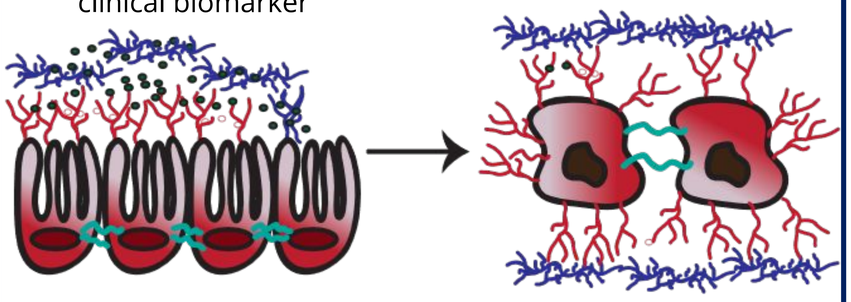
MUCINS

Mucins are a class of proteins whose dense serine- and threonine- bound glycans enforce a bottlebrush-like extended structure.



Mucins are dysregulated in cancer:

- >90% of breast carcinomas upregulate MUC1
- MUC16 (CA125) is highly overexpressed in ovarian cancer and is used as a clinical biomarker

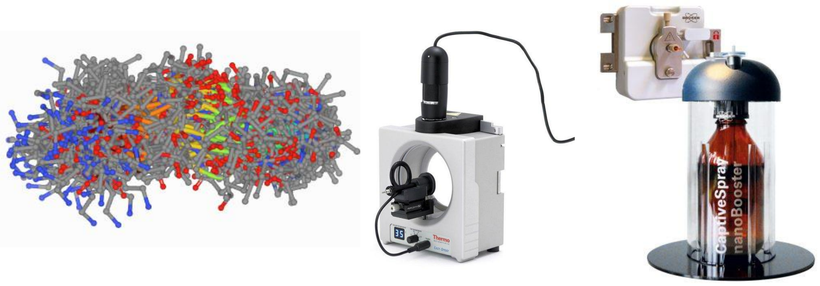


CHALLENGES

- O-glycosylation does not have a **consensus sequence**
- Glycans are non-templated, making the **glycosylation status very diverse and difficult to predict**
- Mucins are characterized by extensive **micro and macro heterogeneity**
- Mucin domains have very dense P/T/S content, rendering the domains **resistant to proteolytic digestion**

IONIZATION

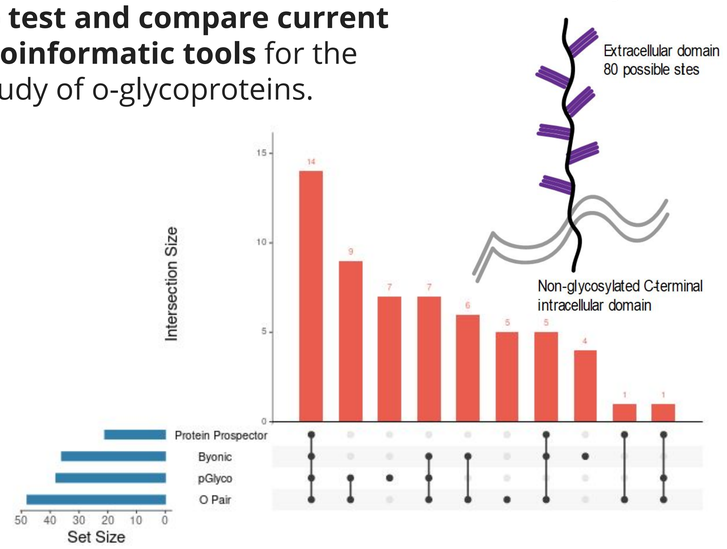
Mucins are characterized by **extremely low ionizability**, given their heavily glycosylated nature. We are interested in modifying current ionization sources, to **increase ionization efficiency** and improve their analysis through MS methods.



BIOINFORMATICS

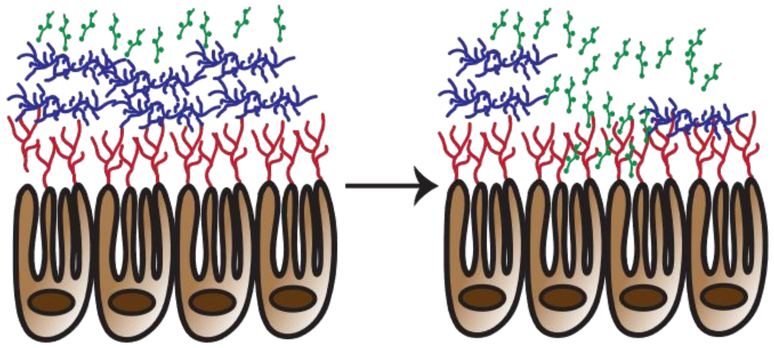
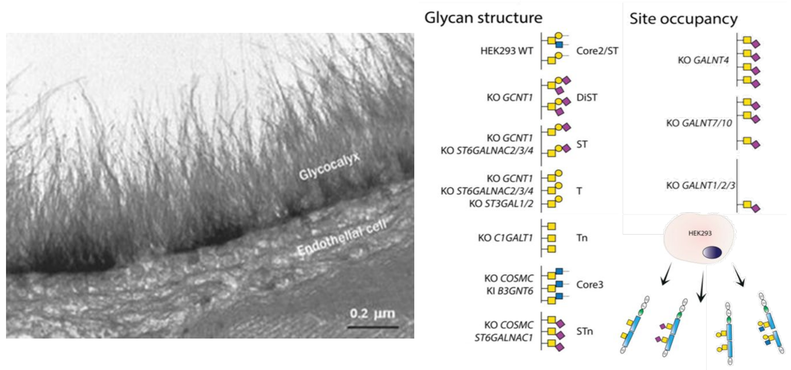
O-glycosylation **requires** electron and collision-based fragmentation methods to characterize glycosites, characterized by very complex spectra. Current bioinformatic platforms **fall short** of the desired performance.

CD43 is **a transmembrane glycoprotein** expressed in a variety of hematopoietic cells, lymphoma, leukemia, and solid tumors. We used CD43 as a **model system**, to **test and compare current bioinformatic tools** for the study of o-glycoproteins.



BIOLOGICAL IMPORTANCE

Mucins in the gut are essential for the **protection of the intestinal epithelium** against commensal and pathogenic bacteria that promote inflammation. When the integrity of the mucin layer is disturbed, chronic inflammation can occur, leading to **inflammatory bowel disease and/or colorectal cancer.**



Mucin-domain glycoproteins are a major component of the **glycocalyx**, the sugary coating surrounding cells. We both characterize glycosylation from **patient samples and glycoengineer cell lines** to better understand mucin domains in health and disease.