In situ deciphering dysregulated protein glycosylation signatures in human ovarian cancer via combined MALDI MS imaging and tandem MS

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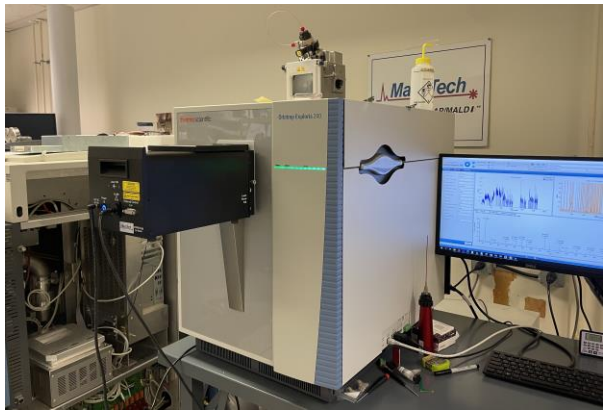
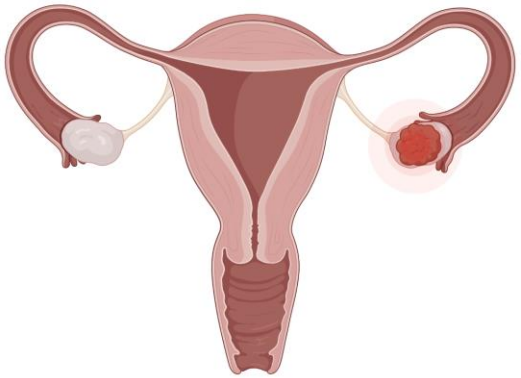
▲ AP-MALDI source on Thermo Orbitrap Exploris 480 (Li Lab)



▲ AP-MALDI source on Thermo Orbitrap Exploris 240 (MassTech Inc.)

Ovarian Cancer

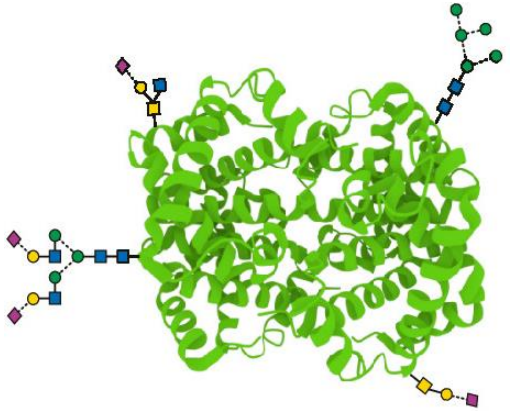


- Each year, approximately 300,000 women worldwide are diagnosed with Ovarian Cancer (OC); 180,000 succumb to this disease.
- Fifth most common cause of female cancer death; leading cause of cancer death in gynecological malignancies.

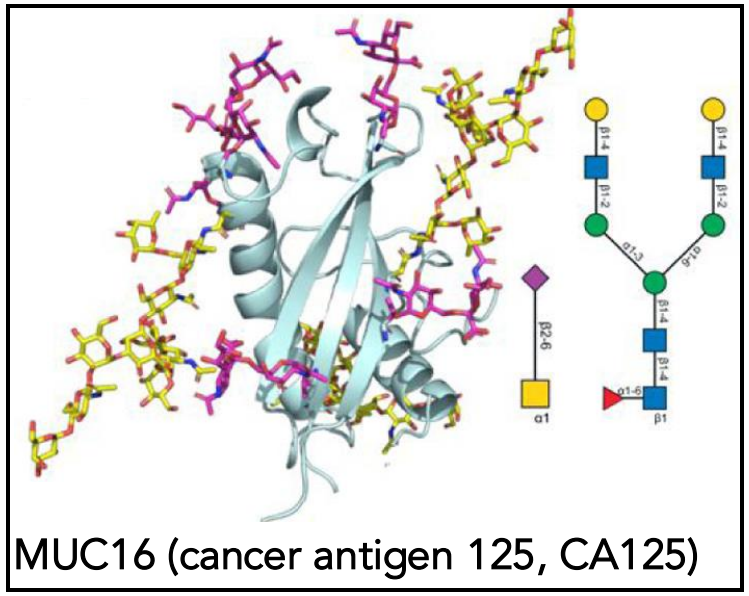
- High mortality rate is due to inadequate early detection.
  - Vague, late-occurring disease symptoms.
  - Lack of early diagnostic markers.
- Patients could have about 90% chance of cure if diagnosed in the early stages.
  - Only 30% of patients are diagnosed with stage I OC.
  - The majority are diagnosed with stage III&IV OC.

1. Grzeski, M. *et al.* In Situ N-Glycosylation Signatures of Epithelial Ovarian Cancer Tissue as Defined by MALDI Mass Spectrometry Imaging. *Cancers (Basel)* **2022**, *14* (4), 1021.
2. Wanyama, F. M.; Blanchard, V. Glycomic-Based Biomarkers for Ovarian Cancer: Advances and Challenges. *Diagnostics (Basel)* **2021**, *11* (4), 643.

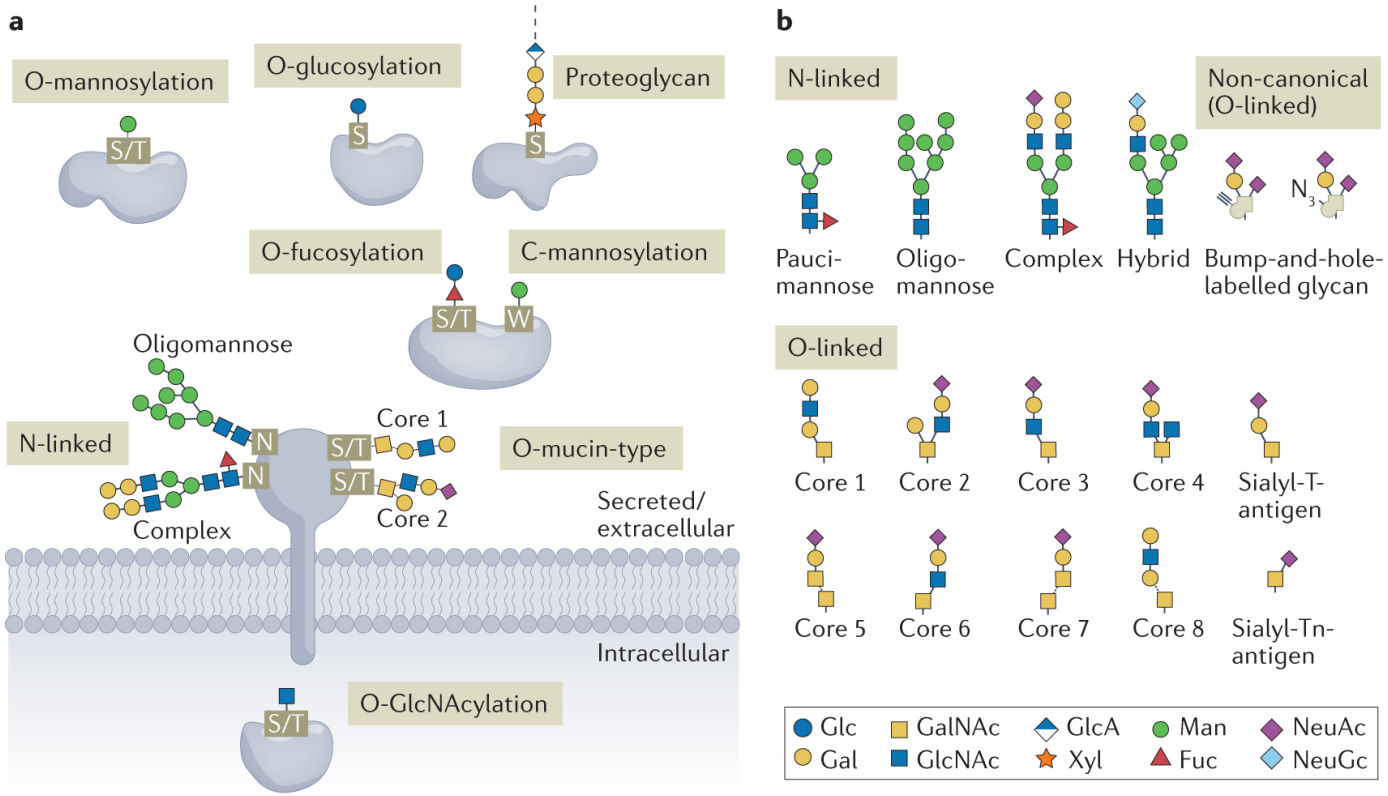
Ovarian Cancer - Glycoproteomics



- Protein glycosylation is one of the post-translational modifications (PTMs) that impact a huge number of biological processes.
- Dysregulation of glycosylation is associated with numerous diseases, such as cancers and Alzheimer's disease.



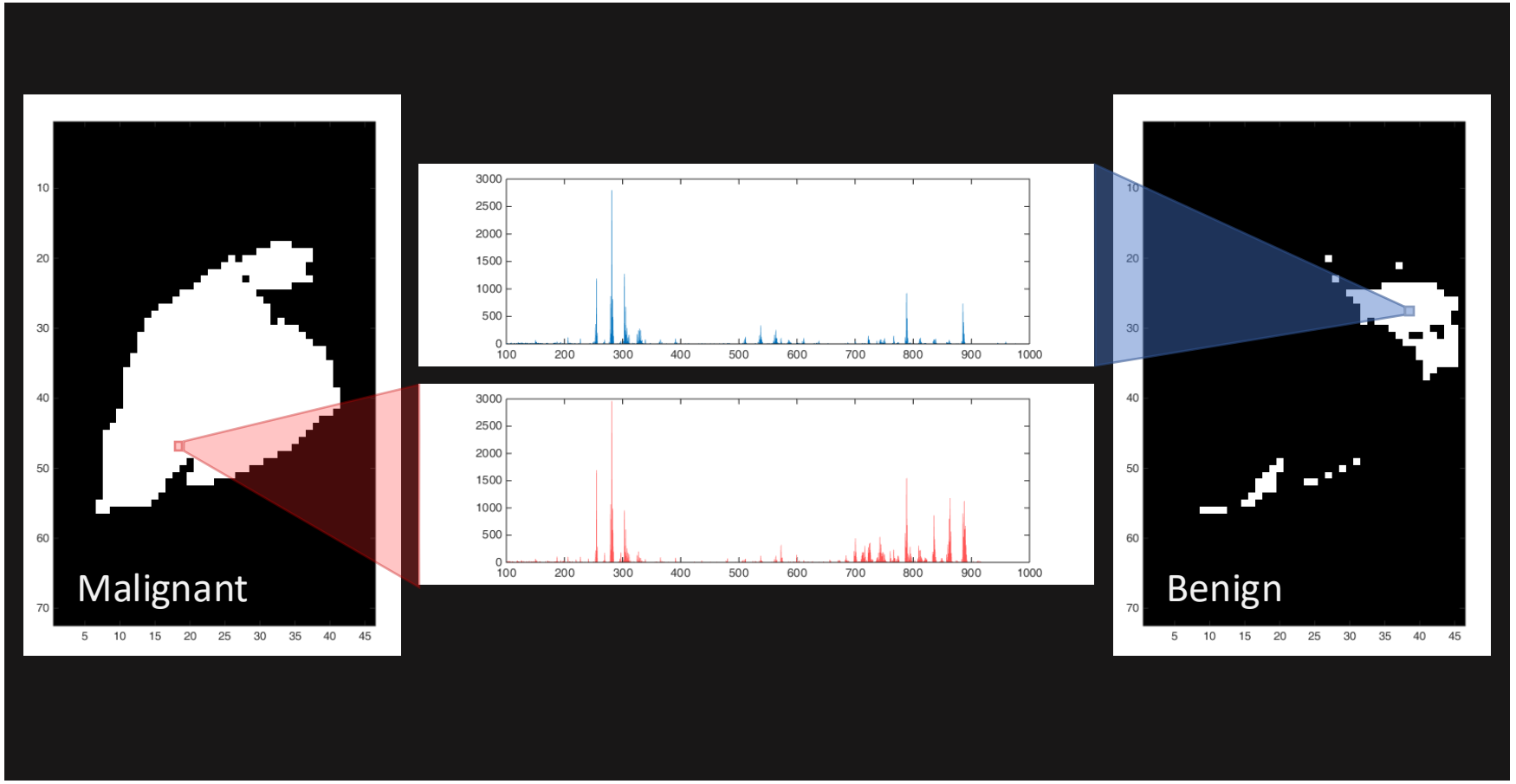
MUC16 (cancer antigen 125, CA125)



1. Bagdonaite, I. *el al.* Glycoproteomics. *Nat Rev Methods Primers* **2022**, *2* (1), 1–29.
2. Grabarics, M.; Lettow, M.; Kirschbaum, C.; Greis, K.; Manz, C.; Pagel, K. Mass Spectrometry-Based Techniques to Elucidate the Sugar Code. *Chem. Rev.* **2022**, *122* (8), 7840–7908.

MALDI Mass Spectrometry Imaging (MSI)

- **Hypothesis:** Protein glycosylation is dysregulated in OC tumor tissues compared to adjacent normal tissues; thus, species detected in malignant tumor regions and benign tumor regions will be different. MSI can serve as a tool to interrogate and visualize the differences.

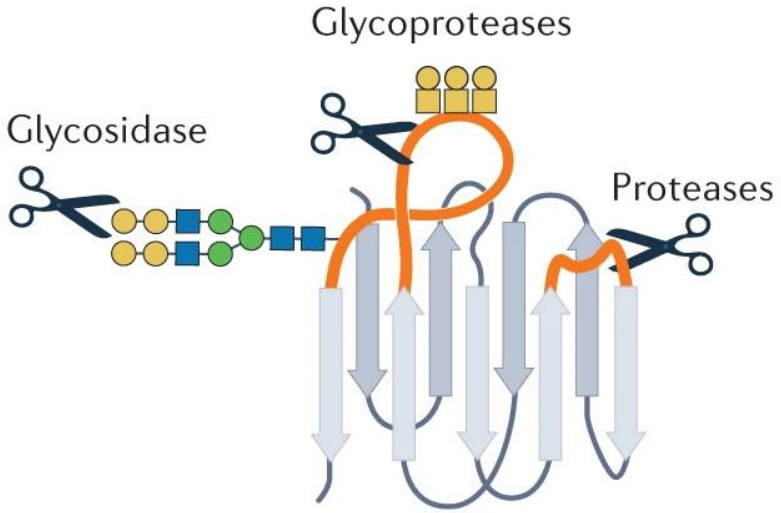


Courtesy of Ying-Chen Huang

Technical Limitation - Glycoproteomics

- Intact glycoproteins are hard to be directly profiled.
  - Large molecules are difficult to be ionized.
  - Relatively low abundant compared to others.

- Glycans and peptides are better starting points to reveal the structures and spatial localization of glycoproteins.
  - The combinational uses of glycosidases and proteases to expand the chemical information obtained from MSI.



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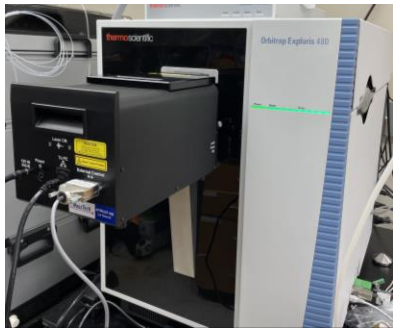
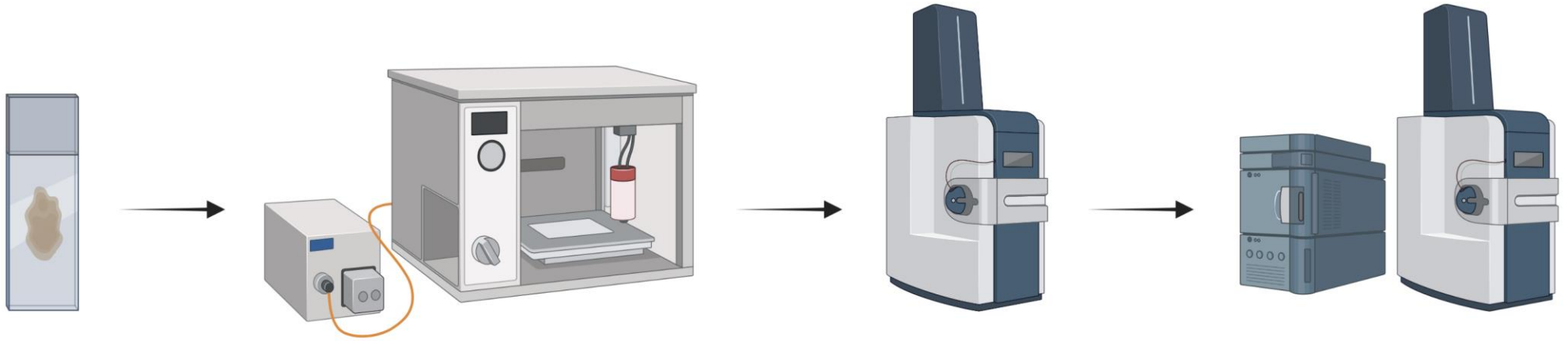
Experimental Workflow

AP-MALDI DDA



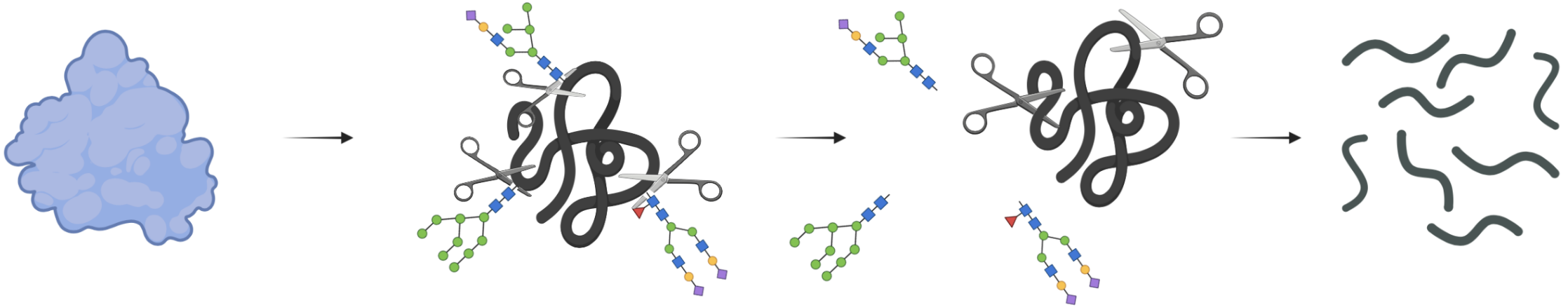


Antigen Retrieval

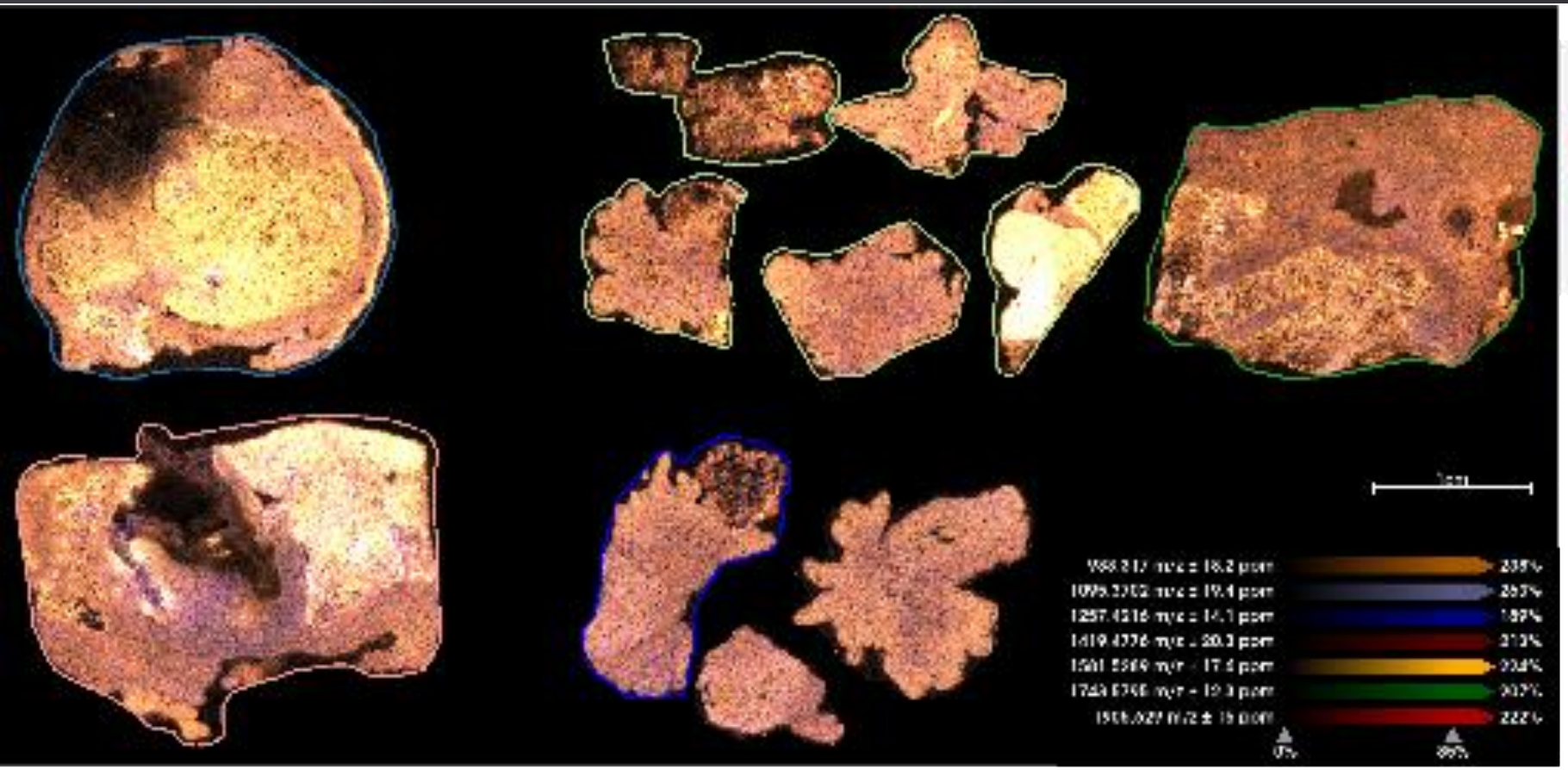
1. Glycosidase (PNGase F)
2. Protease (Trypsin)

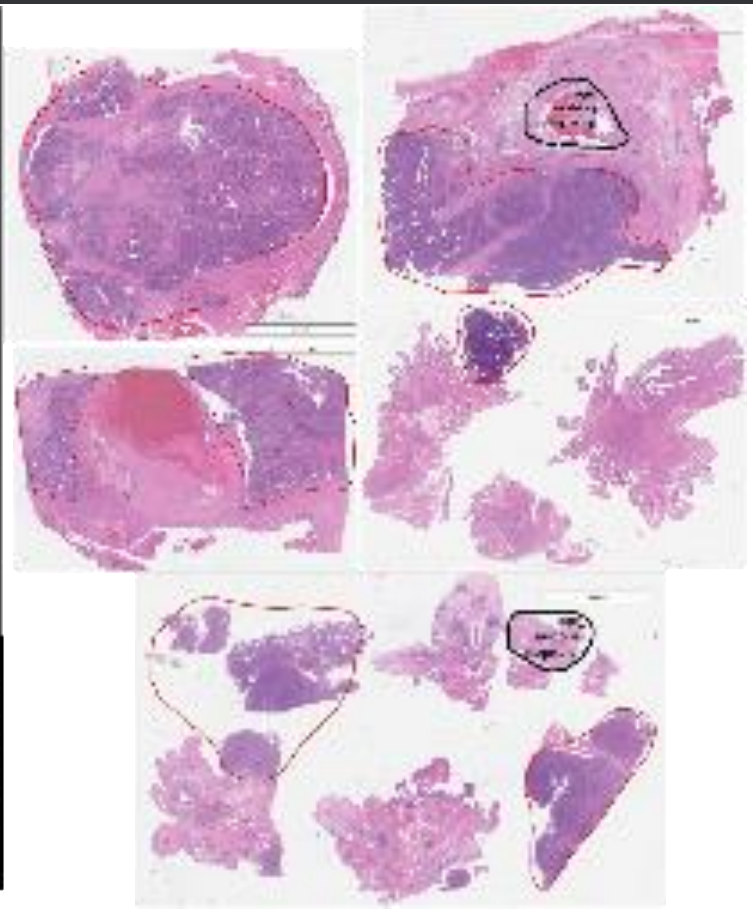
1. Glycan MSI
2. Peptide MSI

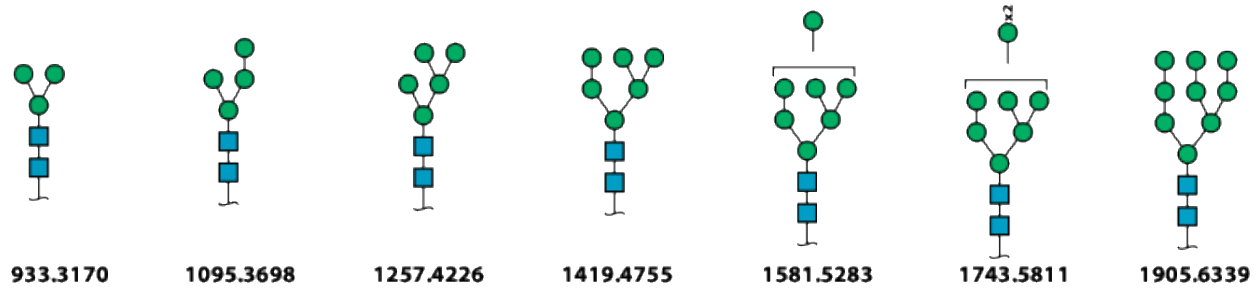
Peptide sequencing of imaged tissues



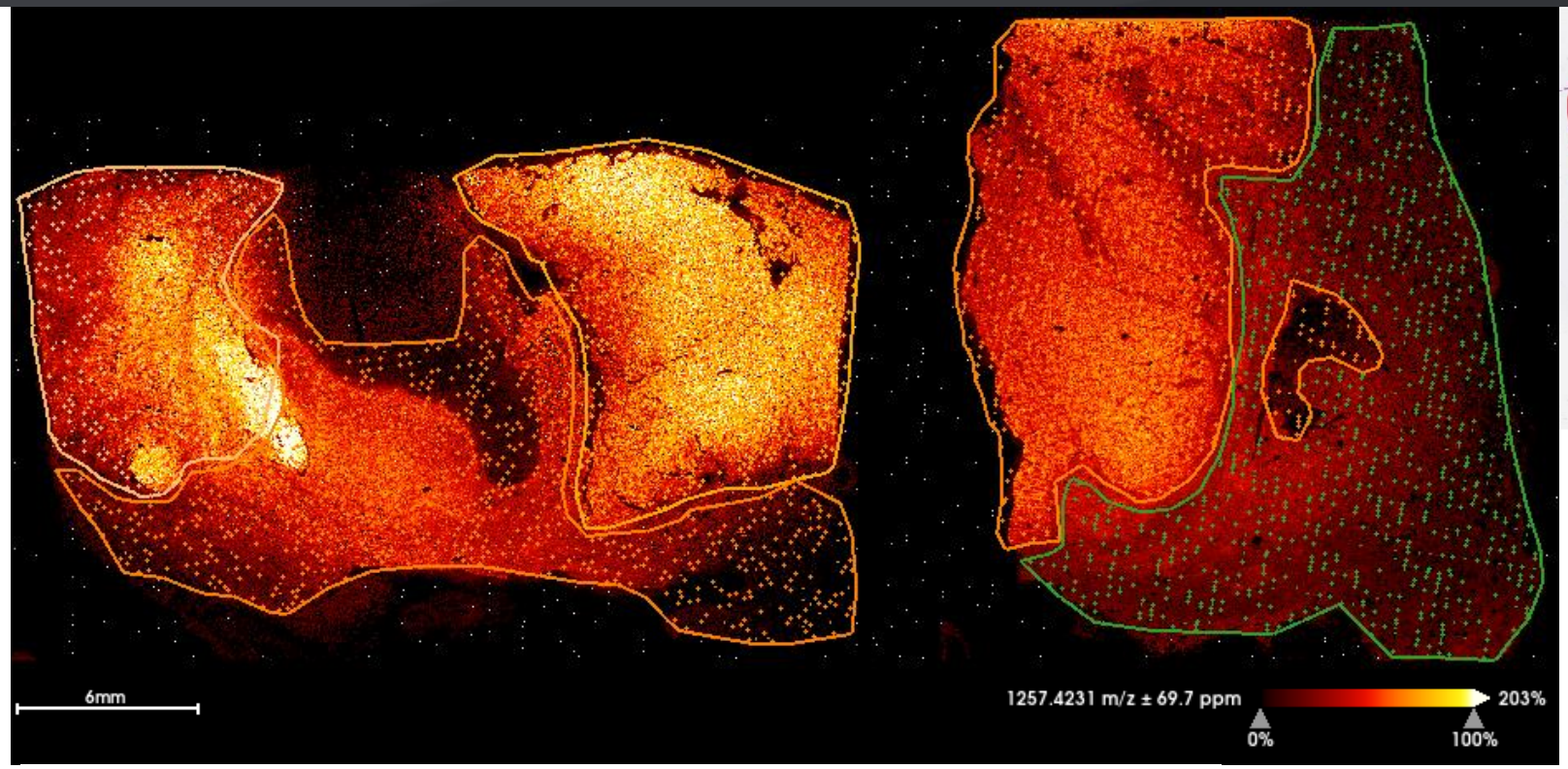
Representative High-mannosylated N-glycans MSI

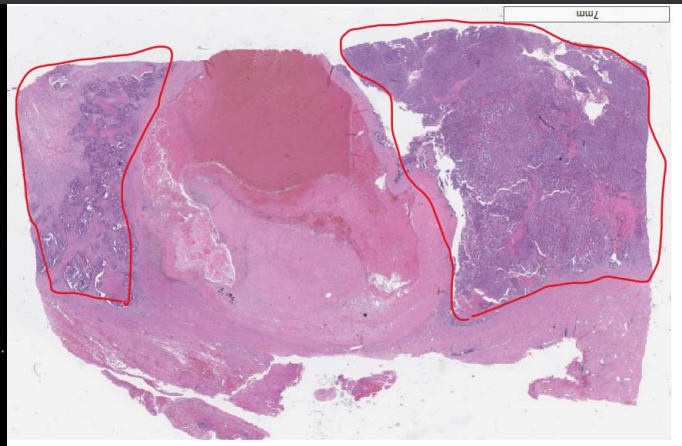






High-mannosylated N-glycans MSI: *m/z* 1257.4





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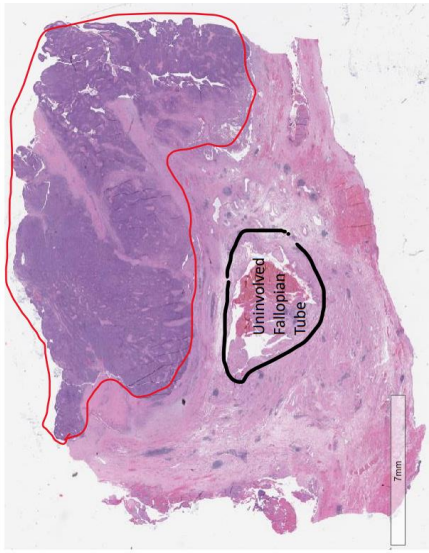

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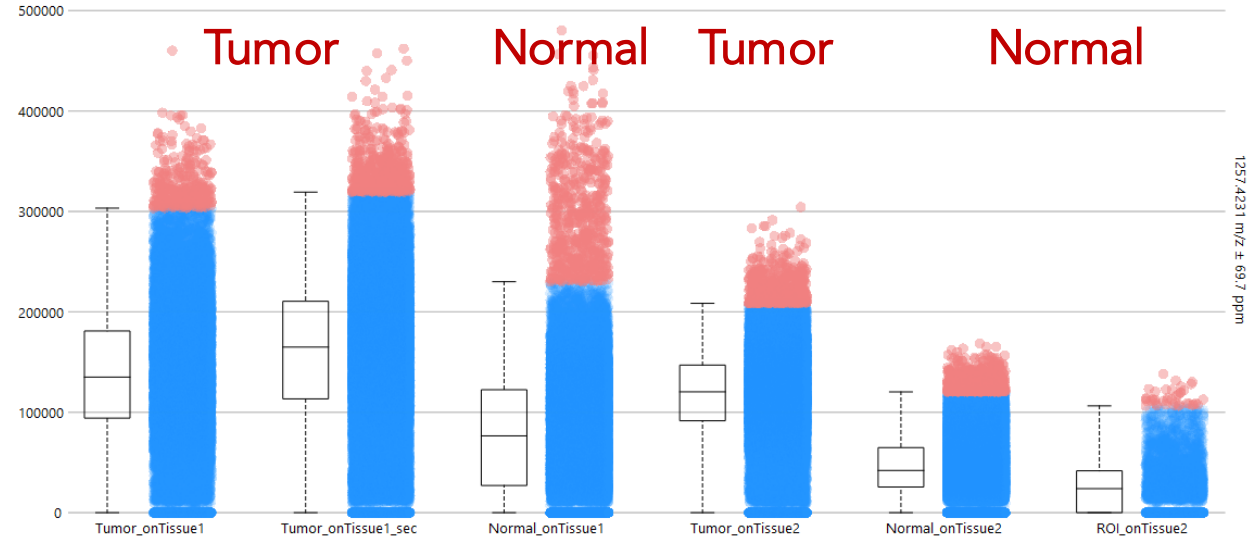
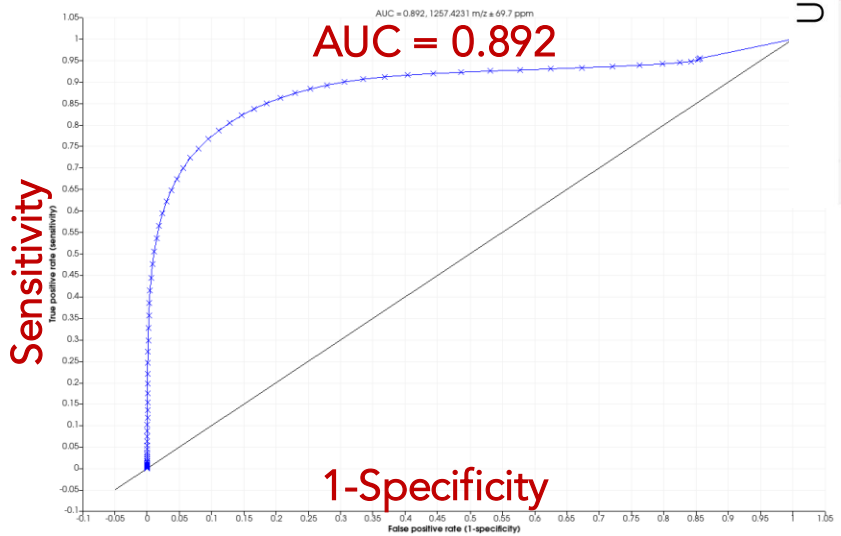
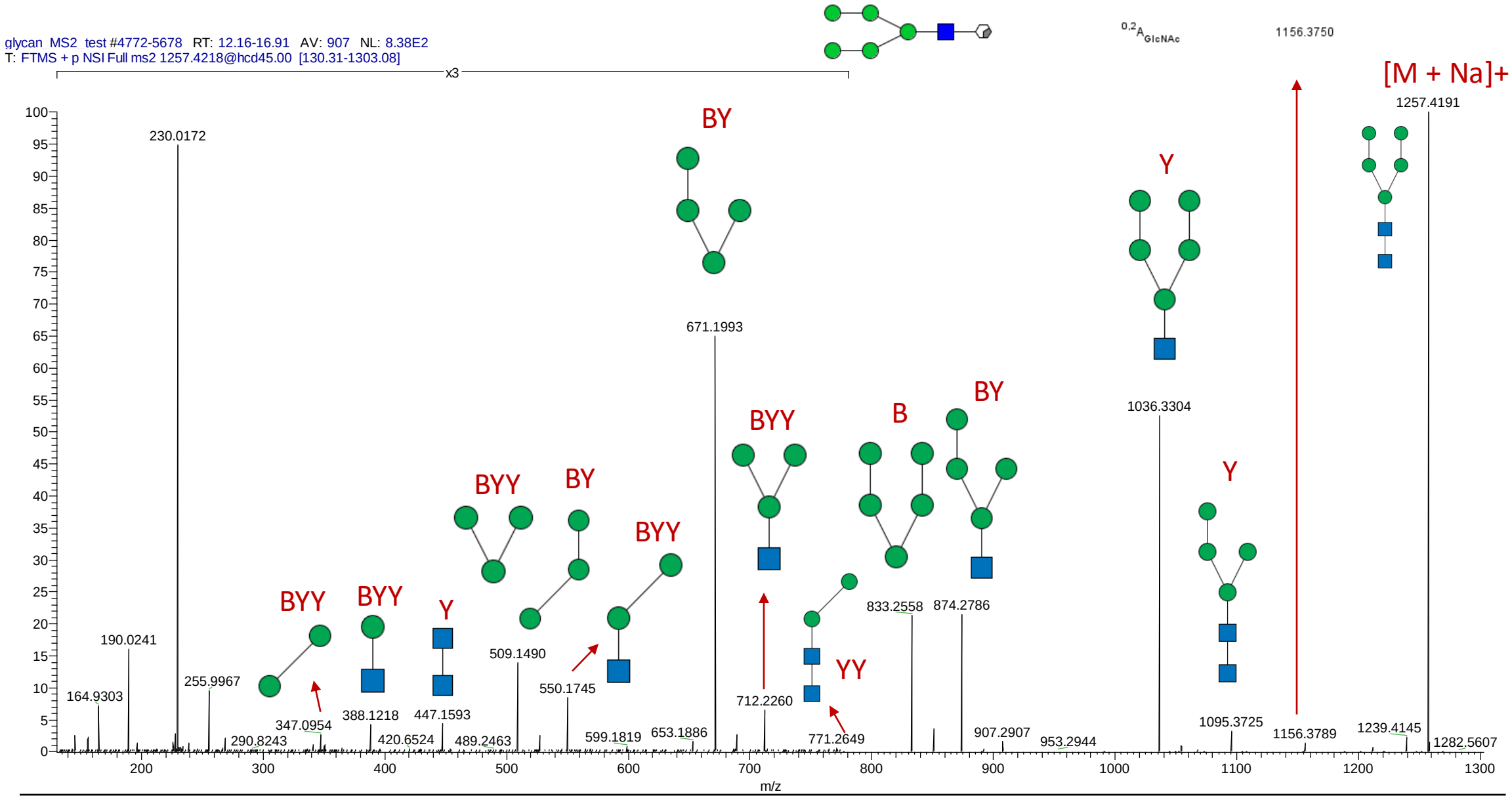
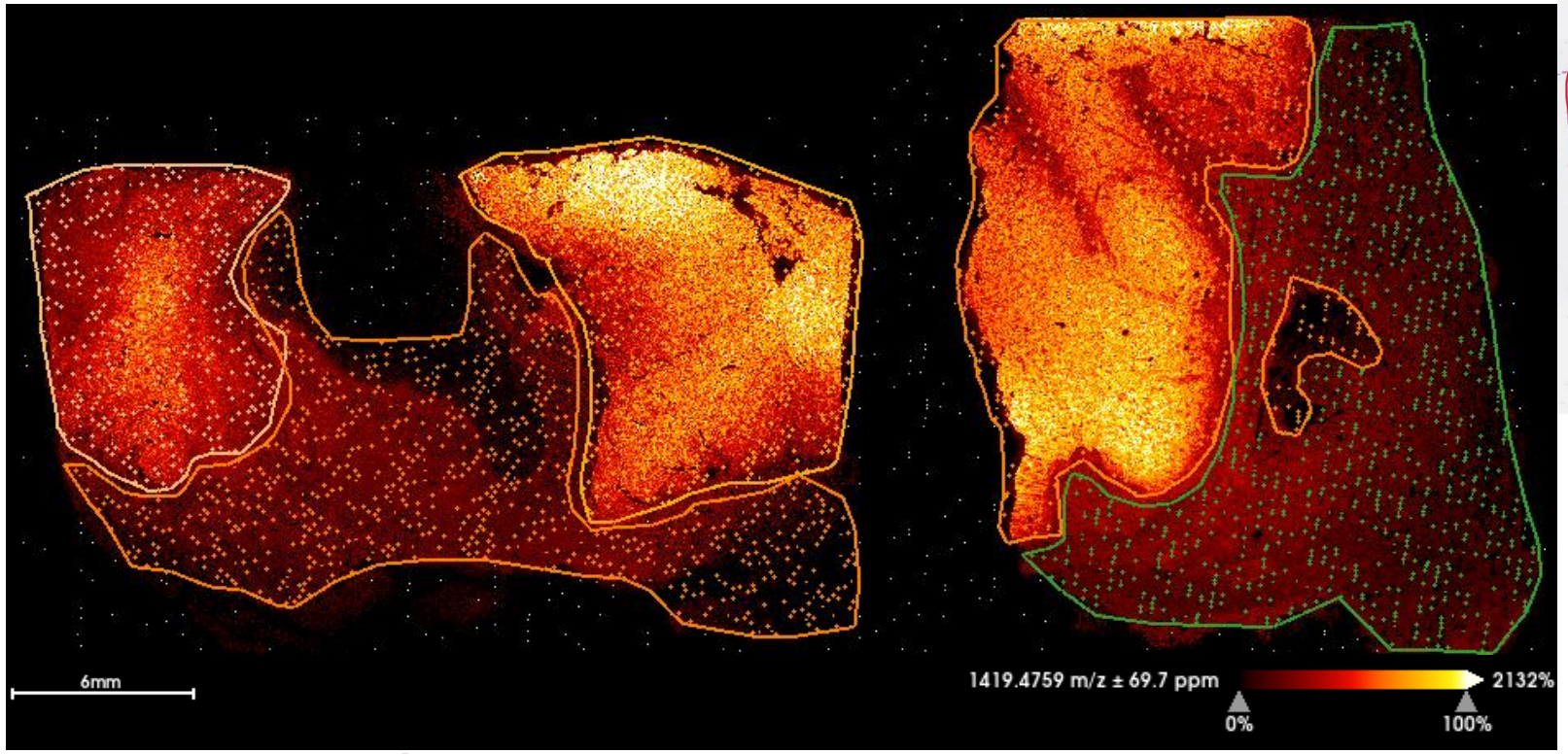


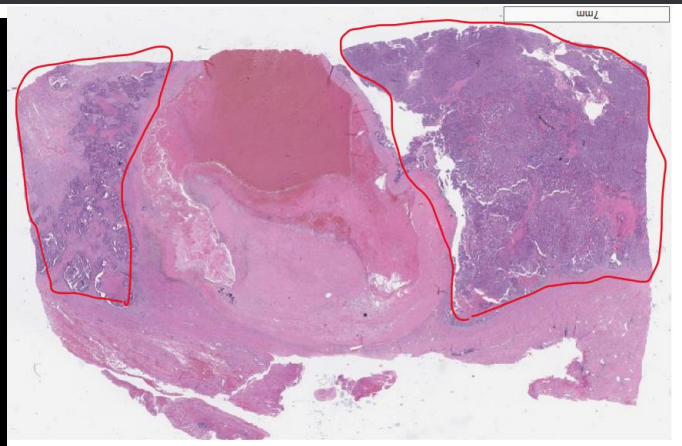


AP-MALDI DDA Characterization of *m/z* 1257.4



High-mannosylated N-glycans MSI: *m/z* 1419.4





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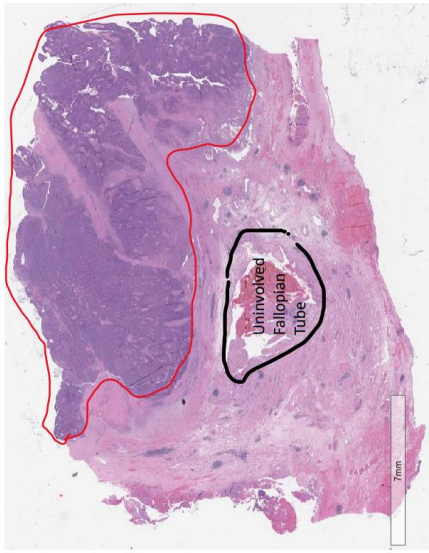

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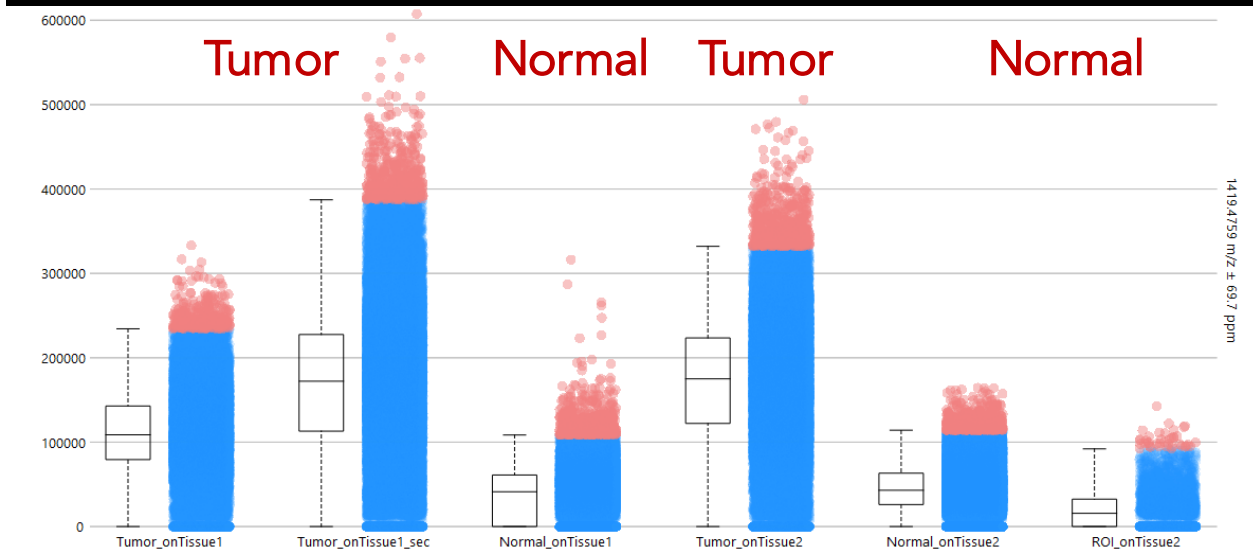
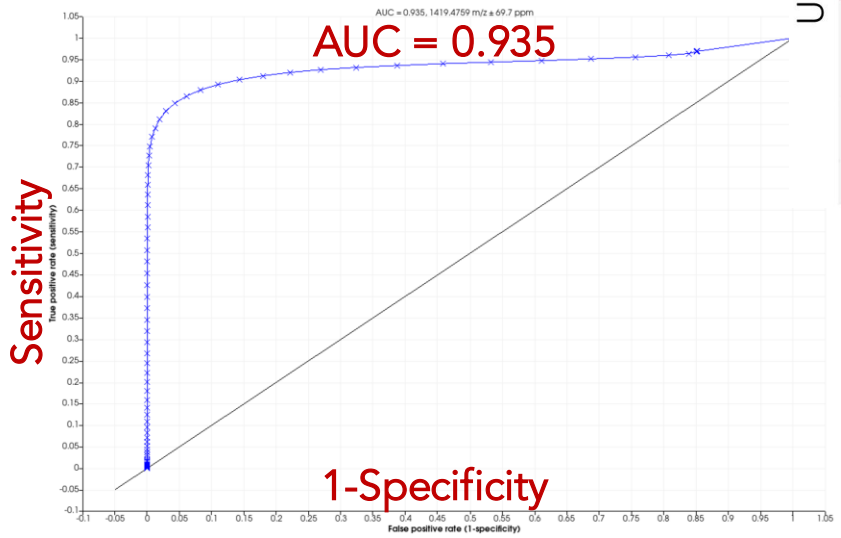
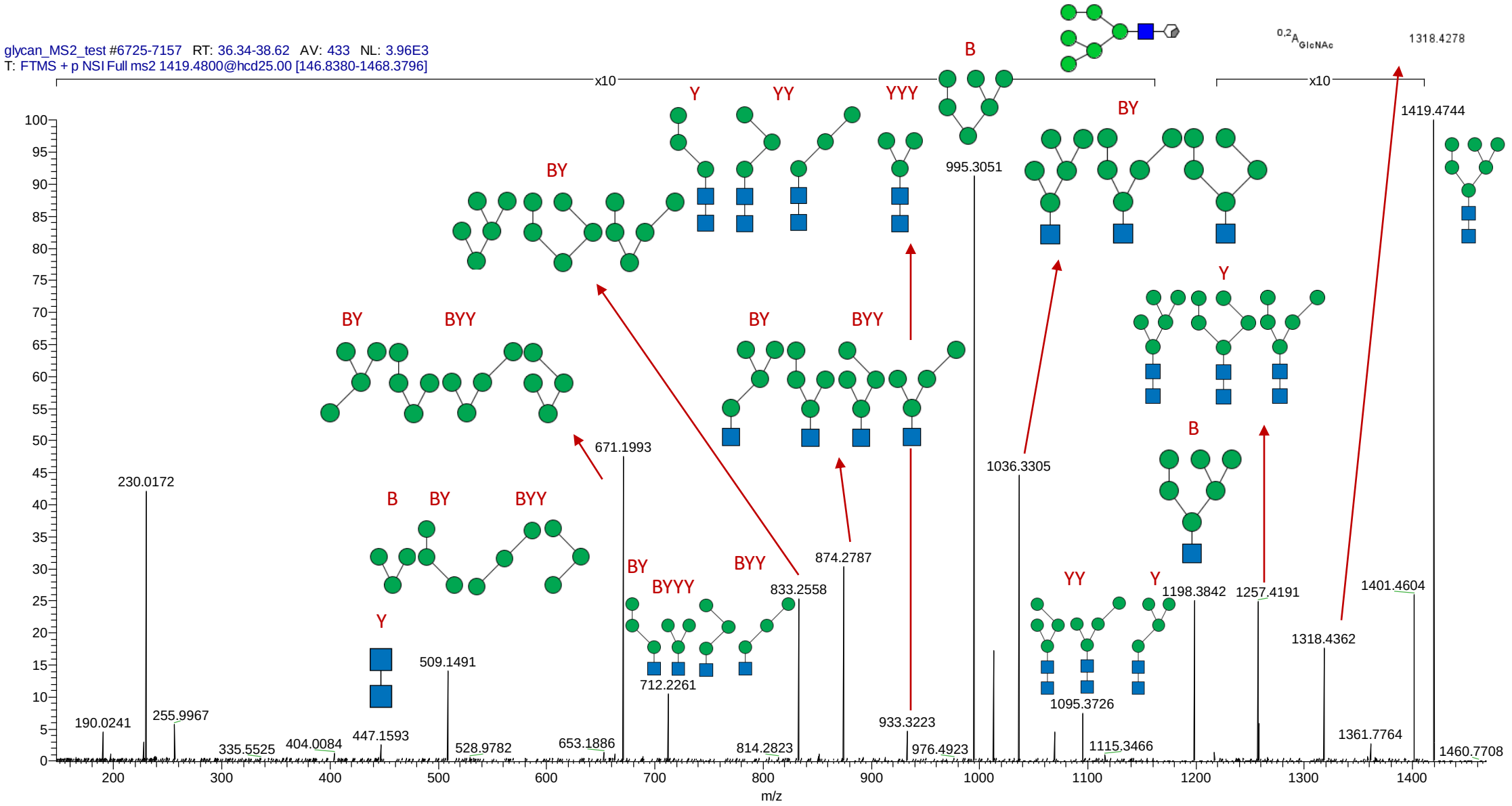
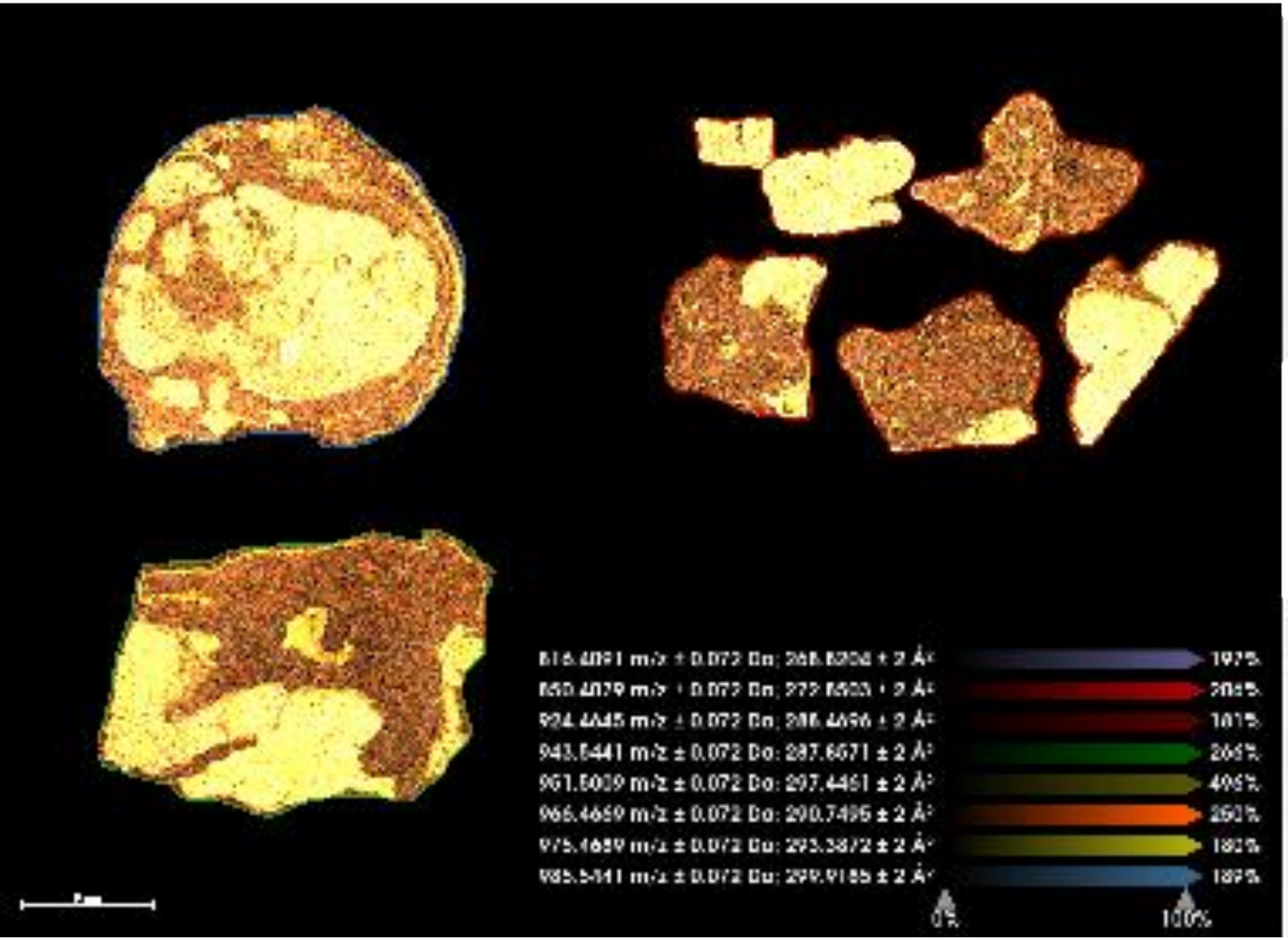


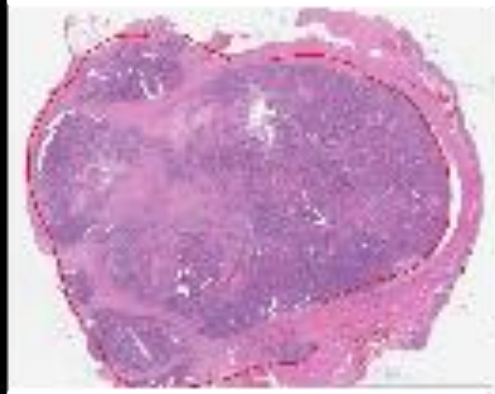


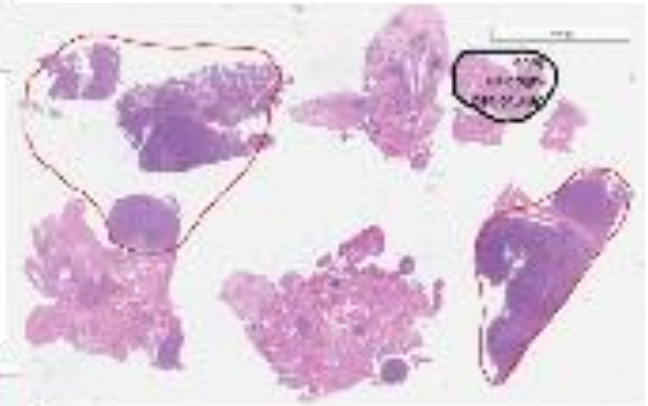
AP-MALDI DDA Characterization of *m/z* 1419.4



Representative putative peptide MSI







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[AAGLLSTYR +H]$^+$ CO1A2_HUMAN
[TVSPALISR +H]$^+$ CAND1_HUMAN
[VVFQEFR +H]$^+$ MYH9_HUMAN
[AGPAGPAGPR +H]$^+$ CO1A2_HUMAN
[VDAATLAR +H]$^+$ DESM_HUMAN

Summary

- Sequential enzymatic digestion to release N-glycans/tryptic peptides to expand chemical coverage on MALDI MSI for biomarker discovery of ovarian cancer.
- On-tissue AP-MALDI DDA-MS^2 characterization of glycan compositions and peptide sequences.
- By combining both LC-MS/MS and AP-MALDI DDA-MS^2, the identifications can be achieved more accurately and confidently *in-situ*.

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Dr. Konstantin Novoselov
Dr. Eugene Moskovets

Poster Presentation – System Biology

ThP 808 Deciphering age-dependent global proteome changes in the leaf-cutting ant Acromyrmex echinatior for better understanding of biomineralization process