COMBINATION OF THE UNIQUE FUNCTIONS FROM timsTOF AND ZenoTOF ENABLES IN-DEPTH ANALYSIS OF THE GLYCOPROTEOME

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INTRODUCTION

- Glycosylation is an abundant and prominent post-translational modification (PTM) found in the serum/plasma proteome; glycoproteomics aims to study protein glycosylation at the site-specific level to reveal the functions of this important PTM
- Many known cancer markers are glycosylated proteins, thus glycoproteomics may provide novel opportunities to identify cancer biomarkers
- PrognomiQ has shown the robustness of timsTOF and ZenoTOF for protein biomarker discovery; however, the potential of these instruments for glycoproteomics has not been fully explored
- This study investigated how to combine the unique features of timsTOF and ZenoTOF to enable in-depth glycoproteomic analysis

OBJECTIVE

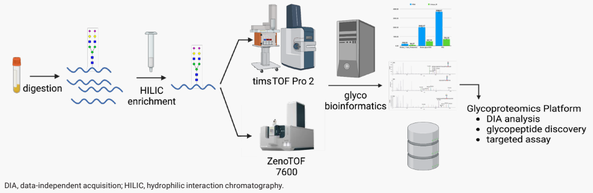
- To develop glycoproteomics methods in timsTOF Pro 2 and ZenoTOF 7600
- To combine the utilities of electron activated dissociation (EAD) from ZenoTOF and ion mobility from timsTOF to gain additional glyco-information
- To generate in-depth glycopeptide library to further biomarker discovery

METHODS

- Pooled plasma (BioIVT) was digested by the iST sample digestion kit from PreOmics
- Glycopeptides were enriched using HILICON iSPE hydrophilic interaction chromatography SPE cartridges; 10 µg plasma equivalent of enriched glycopeptide was used for each injection
- timsTOF Pro 2 coupled with Evosep and ZenoTOF 7600 coupled with a Waters M class LC system were used for data acquisition
- Data were analyzed by Byonic using the 117 *N*-glycans database; a Byonic Score of 250 was used as the cutoff score
- Further analysis was conducted using in-house written R scripts

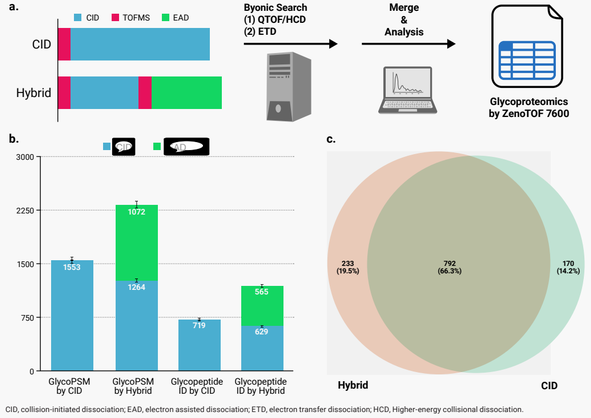
RESULTS

FIGURE 1. Glycoproteomic workflow.



DIA, data-independent acquisition; HILIC, hydrophilic interaction chromatography.

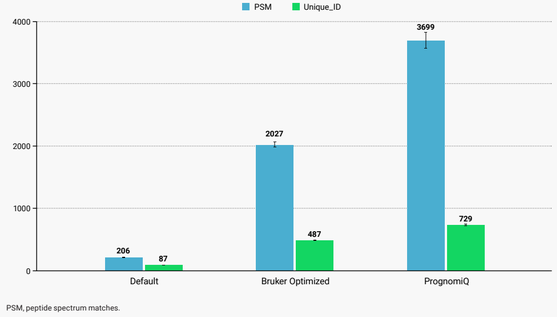
FIGURE 2. A hybrid LC/MS method comprised of both collision-induced dissociation (CID) and EAD increased glycopeptide PSM and IDs.



CID, collision-initiated dissociation; EAD, electron assisted dissociation; ETD, electron transfer dissociation; HCD, Higher-energy collisional dissociation.

- A liquid chromatography/mass spectrometry (LC/MS) method comprised of both CID and EAD MS2 acquisitions was designed and compared with CID-only LC/MS acquisition (Figure 2)
- The resulting raw data were searched by Byonic first with the QTOF/HCD parameter and subsequently with electron-transfer dissociation (ETD) parameters; the results were then merged and analyzed
- In the hybrid mode, the average glycopeptide spectrum matches (PSMs) and IDs in each run were higher compared to the CID-only method
- When condensed to unique glycopeptide IDs, the hybrid mode provided ~20% of IDs that were not identified when using CID-only mode (Figure 2c)

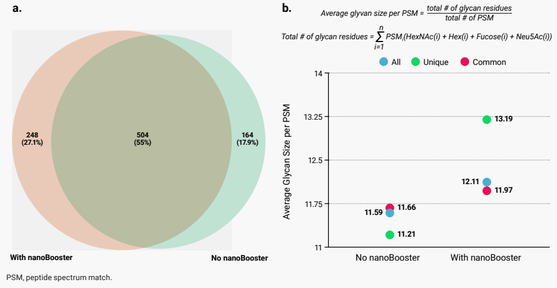
FIGURE 3. Developing a glycoproteomics method in timsTOF Pro 2.



PSM, peptide spectrum matches.

- Stepped collision energy (SCE), along with other settings, were optimized to increase the number of unique glycopeptide IDs
- Compared to Bruker's glycoproteomics method, our optimized method had an 82.5% increase in PSMs and a 49.7% increase in unique glycopeptide IDs (Figure 3)

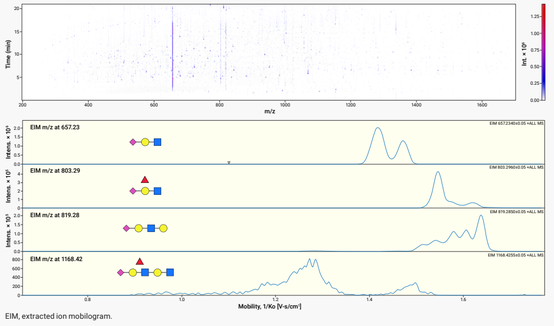
FIGURE 4. nanoBooster enabled identification of glycopeptides with larger glycans.



PSM, peptide spectrum match.

- Glycopeptides identified with or without nanoBooster were analyzed (Figure 4)
- The nanoBooster provided an additional 27% of IDs compared to when no nanoBooster was used (Figure 4a)
- Glycopeptides uniquely identified using nanoBooster carried larger glycans (Figure 4b)

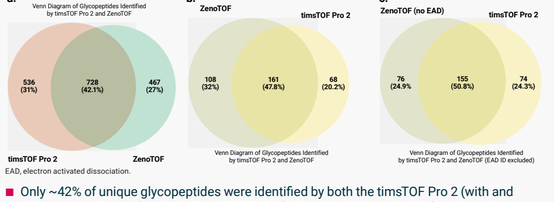
FIGURE 5. timsTOF ion mobility elucidated additional structural information of glycopeptides.



EIM, extracted ion mobilogram.

- The heatmap of *m/z* over retention time showed ions presented across the whole retention time (Figure 5)
- These ions were found to be in-source fragmented glycan oxonium ions, and extracted ion mobilogram showed separation of these oxonium ions
- These results suggest that additional glycan structural information could be elucidated from the ion mobility data

FIGURE 6. timsTOF and ZenoTOF see glycoproteome differently.



EAD, electron activated dissociation.

- Only ~42% of unique glycopeptides were identified by both the timsTOF Pro 2 (with and without nanoBooster) and the ZenoTOF 7600 (CID and hybrid acquisition), and each instrument provided ~30% unique glycopeptides (Figure 6a)
- Results suggest that glycoproteome coverage can be enhanced through the unique capabilities of each instrument
- When comparing stripped peptides, the overlap between ZenoTOF and timsTOF Pro 2 increased to ~48%, suggesting the 2 instruments see glycan structures differently (Figure 6b)
- When EAD data from ZenoTOF were excluded, the overlap increased further to 50.8%, suggesting some peptides were uniquely identified by the EAD feature in ZenoTOF (Figure 6c)

CONCLUSIONS

- Combined CID and EAD increased glycopeptide IDs with a single acquisition in ZenoTOF 7600 system
- SCE optimization was critical to glycopeptide identification
- NanoBooster allowed for the identification of large glycopeptides in timsTOF Pro 2
- timsTOF ion mobility elucidated additional structural information of glycopeptides
- Optimization of the LC/MS method and the combination of unique features of the 2 platforms enhanced coverage of the glycoproteome

DISCLOSURES:
Study funded by PrognomiQ, Inc. All authors are current or former employees of PrognomiQ, Inc.

ACKNOWLEDGEMENTS:
Funded by PrognomiQ, Inc (San Mateo, CA). Editorial and graphical assistance provided by Prescott Medical Communications Group (Chicago, IL)