Multi-Attribute Method reporting with Panorama and Skyline

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Overview

Panorama is a web-based data management system for targeted mass spectrometry data. It integrates closely with the popular Skyline desktop analysis tool. The Multi-Attribute Method (MAM) is designed for improved simultaneous detection, identification, quantitation, and quality control (monitoring) of molecular attributes. Panorama has recently added MAM-specific reporting to its existing set of analytics, offering immediate results and data sharing to any imported data. Additionally, Panorama and AutoQC's automated workflow provide longitudinal tracking of MAM-related metrics for QC purposes.

Introduction

Create Skyline document

- Skyline, a Windows application, supports all major mass spec vendors
- The document captures the peptides, transitions, and modifications to be monitored

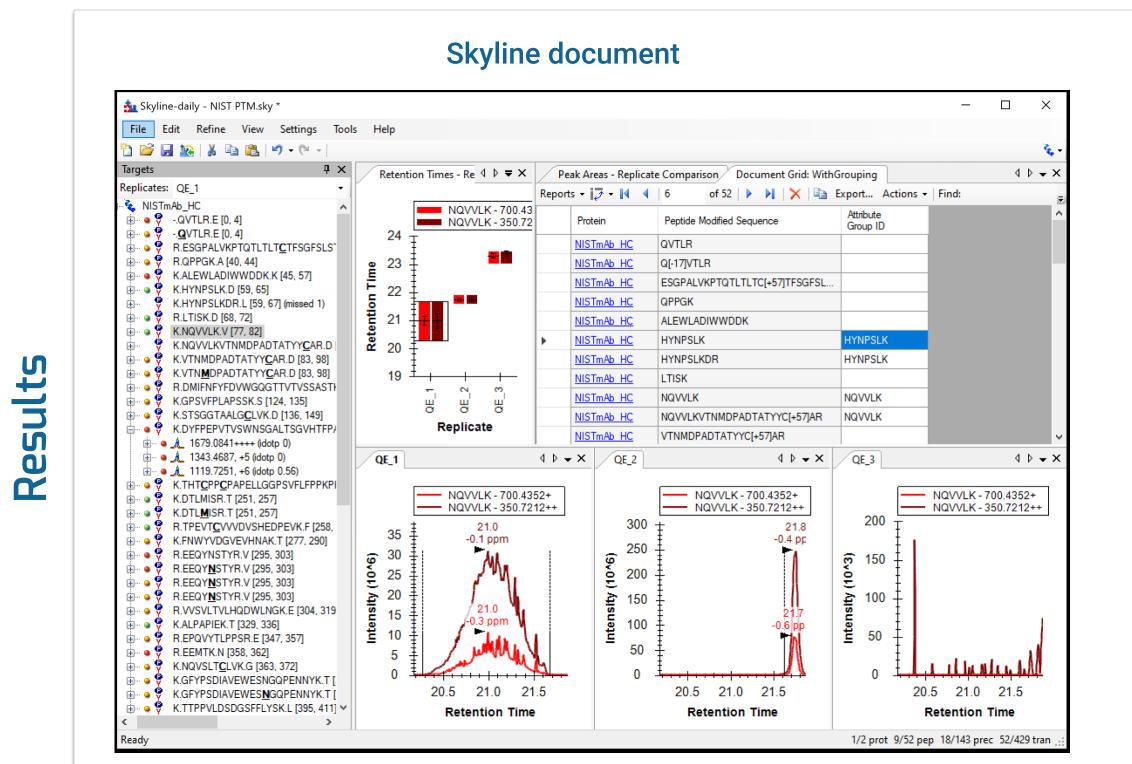
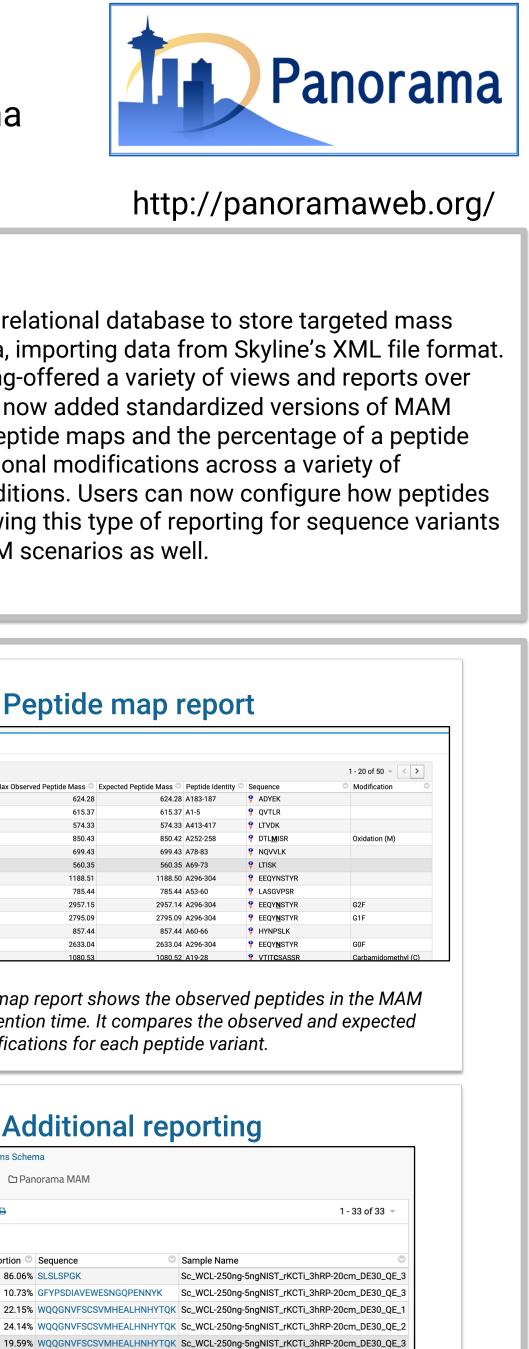


Figure 1: Skyline lets users easily set up the proteins and peptides to analyze, and pull in raw data from all of the major mass spec vendors without needing to do a separate conversion step. Here, a Skyline document shows a reference NIST monoclonal antibody (Dong et al) results as analyzed on a Orbitrap Lumos (Levy et al). The document grid in the upper right includes the Attribute Group ID column, which lets users associate peptide variants that do not share a common unmodified sequence. Here, the user has manually grouped four peptides into two different groups to capture missed cleavages as a single group.

Conclusions

Groups first started creating MAM-related reports in Panorama via custom SQL queries in 2015, but these were not built-in as standard reports or widely distributed. As interest in MAM has grown, the Panorama team saw the need to generalize them and make them broadly available. The initial set of reports include post-translational modification percent and peptide map reports. A collaboration between users and the Skyline and Panorama development teams resulted in new capabilities to override how peptides and their variants are grouped when calculating the percentage that each variant represents of the whole. This flexible approach means that users can either use the default, which groups all PTM variants together, or group based on sequence variants or other factors. Supporting these reports directly also means that database schema changes and precalculation can speed up query times by 20x or more. Additionally, because Panorama can track a wide variety of data for automated longitudinal tracking, these MAM-related metrics can be easily incorporated into QC folders where they can be analyzed with statistical process control techniques like Levey-Jennings, CUSUM, and moving range plots. As of January 2020, more than 400 labs are using Panorama projects free of charge to manage targeted mass spectrometry assays on http://panoramaweb.org/, a server hosted by the MacCoss lab at the University of Washington. Additionally, major pharmaceutical companies and other organizations have deployed their own in-house installations of Panorama.



Set up Panorama folder

- Panorama organizes data into folders, typically one per instrument being monitored
- A new MAM-oriented variant includes predefined reports that are unique to MAM analysis

Analyze and integrate results

- Web-based interface is ideal for sharing collaborators, including non-Skyline users
- Results can be easily leveraged elsewhere, such as Spotfire visualizations or tracking via Panorama's system suitability monitoring

Methods

Panorama uses a relational database to store targeted mass spectrometry data, importing data from Skyline's XML file format. Panorama has long-offered a variety of views and reports over this data, and has now added standardized versions of MAM reports such as peptide maps and the percentage of a peptide with post-translational modifications across a variety of experimental conditions. Users can now configure how peptides are grouped, allowing this type of reporting for sequence variants and other non-PTM scenarios as well.

Panorama Multi-Attribute Method folder

Configure Panorama Folder: /Panorama MAM

Users / Permissions Project Settings	Chromatogram library - Curated precursor and product ion expression data for use in designing and validating future experiments
Change File Root	O Quality control - System suitability monitoring of reagents and instruments
Configure Panorama Folder	
Configure Panorama Folder Document Summa	ry

Figure 2: Panorama organizes data into folders, and offers different folder types that are optimized for different workflows and analyses. A new MAM folder type introduces predefined reports guides the user to them through links in the Document Summary view.

Post-translational modification report

PTM Report							
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					Sample Name		
				Sc_WCL-250ng- 5ngNIST_rKCTi_3hRP- 20cm_DE30_QE_3	Sc_WCL-250ng- 5ngNIST_rKCTi_3hRP- 20cm_DE30_QE_2	Sc_WCL-250ng- 5ngNIST_rKCTi_3hRP 20cm_DE30_QE_1	
Modification	Sequence	Site Location	Chain	\odot	\odot	C	
Oxidation (M)	R.VGYMHWYQQKPGK.A	M32	NISTmAb_LC	13.59%	14.62%	17.89%	
Oxidation (M)	K.VTNMDPADTATYYCAR.D	M87	NISTmAb_HC	12.11%	11.15%	11.319	
Oxidation (M)	R.WQQGNVFSCSVMHEALHNHYTQK.S	M431	NISTmAb_HC	19.59%	24.14%	22.15%	
Oxidation (M)	💡 K.DTL <u>M</u> ISR.T	M255	NISTmAb_HC	44.55%	38.44%	41.029	
Oxidation (M)	DIQMTQSPSTLSASVGDR.V	M4	NISTmAb_LC	9.94%	9.85%	9.41%	
Gln->pyro-Glu (N-term Q)	💡QVTLR.E	Q1	NISTmAb_HC	99.58%	99.54%	99.339	
G2F	R.EEQYNSTYR.V	N300	NISTmAb_HC	4.18%	3.66%	3.80%	
G1F	💡 R.EEQY <u>N</u> STYR.V	N300	NISTmAb_HC	44.20%	40.65%	43.419	
G0F	R.EEQYNSTYR.V	N300	NISTmAb_HC	45.14%	46.58%	48.80%	
Deamidated (NQ)	K.GFYPSDIAVEWES <u>N</u> GQPENNYK.T	N387	NISTmAb_HC	10.73%	6.61%	7.39%	
Lys clipping	K.SLSLSPGK	K450	NISTmAb HC	86.06%	85.98%	84.96%	

Figure 3: The post-translational modification (PTM) report shows the proportion for each peptide variant's peak area across samples. Panorama automatically groups peptides with identical unmodified sequences, but a user can configure alternative groupings within the Skyline document, to group splice variants, for example.

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Retention Time 🗕 🛇	Min Observed Peptide Mass 📀	Max Observed Peptide Mass 📀	Expected Peptide Mass 📀	Peptide Identity 🛇	Seque
10.5	624.28	624.28	624.28	A183-187	💡 AD'
13.2	615.37	615.37	615.37	A1-5	💡 QV
14.6	574.33	574.33	574.33	A413-417	💡 LTV
21.4	850.43	850.43	850.42	A252-258	💡 DTI
22.0	699.43	699.43	699.43	A78-83	💡 NQ'
22.3	560.35	560.35	560.35	A69-73	💡 LTIS
23.4	1188.51	1188.51	1188.50	A296-304	💡 EEC
25.1	785.44	785.44	785.44	A53-60	💡 LAS
26.7	2957.14	2957.15	2957.14	A296-304	💡 EEC
26.8	2795.09	2795.09	2795.09	A296-304	💡 EEC
26.8	857.44	857.44	857.44	A60-66	💡 HY
27.3	2633.04	2633.04	2633.04	A296-304	💡 EEC
29.2	1080.52	1080.53	1080.52	A19-28	💡 VTI

Figure 4: The peptide map report shows the observed peptides in the MAM analysis, sorted by retention time. It compares the observed and expected masses, and the modifications for each peptide variant.

Additional reporting

Query Schema	Browser / targetedms Scher	na	
PTMPerc	entsPrepivot 🗅 Pan	orama MAM	
	≝ - ± - ⊖		
🖽 Flattene	d export		
Chain ©	Modified Area Proportion ©	Sequence	Sample Name
NISTmAb_HC	86.06%	SLSLSPGK	Sc_WCL-250ng-5ngN
NISTmAb_HC	10.73%	GFYPSDIAVEWESNGQPENNYK	Sc_WCL-250ng-5ngN
NISTmAb_HC	22.15%	WQQGNVFSCSVMHEALHNHYTQK	Sc_WCL-250ng-5ng
NISTmAb_HC	24.14%	WQQGNVFSCSVMHEALHNHYTQK	Sc_WCL-250ng-5ngN
NISTmAb_HC	19.59%	WQQGNVFSCSVMHEALHNHYTQK	Sc_WCL-250ng-5ngN

Figure 5: The Panorama team is adding more MAM-focused reporting. Additionally, because Panorama uses a relational database for its storage, the data is easily presented in alternative formats, integrated with other data sources, and made available to other tools. For example, PTM percentages can be used as a metric in a Panorama QC folder for system suitability monitoring. Data can be pulled into Spotfire, Tableau, or R for additional visualization.

References

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