

Summary

Comparison of six different strategies for label-free peptide quantitation, based on protease and charge state selection

A hybrid approach of two of the assessed method shows superiority

Implementation of this method in Protein Metrics' Byos[®] software for automated data processing from the raw file to the report

Introduction

- Post-translational modifications (PTMs) play a pivotal role in the development of biopharmaceuticals, adding complexity and diversity to protein structures, that can considerably impact a protein's function, stability, and pharmacokinetics
- Suitability of six different strategies for quantitation of PTMs in therapeutic proteins have been assessed and are summarized in the table below

Nr.	Short Name	WT Intensity Threshold	Charge States	Digests	Weighted Quantitation
1	Trypsin (all z)	Most Intense	All+WTs without Mod	Trypsin	NA
2	Trypsin (most intense z)	Most Intense	Most Intense	Trypsin	NA
3	Most Intense	Most Intense	Most Intense	All	NA
4	Mean>1e7	1e7	All	All	No
5	Weighted Specific Enzymes	All	All	Specific Enzymes	Yes
6	Weighted All Enzymes	All	All	All	Yes

- The selection of the peptides that contain the modified residue is challenging due to factors like multiple charge states, low signal intensities and missed cleavage sites
- The picture below shows the selection of peptides for the quantitation strategy Nr. 4



Results

- Each quantitation strategy was evaluated against four different factors:
 - SVs(%): Percentage of captured sequence variants
 - 5-fold lower quant: fivefold underestimation of the sequence variant abundance
 - 5-fold upper quant: fivefold overestimation of the sequence variant abundance
 - Mean Deviation to 1%: The mean deviation from the known abundance of 1%

	Trypsin (most intense z)	Trypsin (all z)	Most Intense	Mean > 1e7	Weighted Specific Enzymes	Weighted All Enzymes
SVs (%)	81	81	100	100	91	100
5-fold lower Quant	7	8	17	0	1	0
5-fold upper Quant	3	3	2	18	3	8
Mean Deviation to 1%	4.3	8.2	19.4	2.8	2.3	2.3

- The study concludes that a hybrid approach, incorporating elements from strategies 5 & 6 (Weighted Specific Enzymes and Weighted all Enzymes), is most effective for accurate quantitation
- If a multi enzyme approach is selected and the peptides of the specific enzyme digests are not covering the whole sequence, those gaps can be filled with non-specific digests

Implementation in Byos

- Byos from ProteinMetrics is a software solution for the identification and quantitation of peptides analyzed with LC-MS
- The report capabilities of the software enable the implementation of the quantitation strategy (hybrid of the strategies 5 & 6) as shown in the picture below

Quantitation Key	Med. Summary	Sequence ↑	MS Alias Name	VL230802_05_Nist_TR (%)
		K.GFYPSDIAVEWESNGQPENNYK.T		96.02
gi 002 NISTHC NIST_REFSTD_HC;N392,W384,N393,N387	N14(Asn-Succinimide/-17.0265)	K.GFYPSDIAVEWESnGQPENNYK.T		1.28
	N19(Deamidated/0.9840)	K.GFYPSDIAVEWESNGQPENNYK.T		2.42
	N20(Asn-Succinimide/-17.0265)	K.GFYPSDIAVEWESNGQPENNYK.T		0.25
	W11(Trp-Kynurenin/3.9949)	K.GFYPSDIAVEWESNGQPENNYK.T		0.04

Normalize Column - Total XIC AUC Averagine, Level 1

Normalize type - Sum

Render type: Col Heatmap

Color by: Total XIC AUC Averagine

Filter Applied on: CustomKey, CustomKeyType, Mod. Summary, Quantitation Key

- All peptides containing the same modified residue are grouped independently of the utilized enzyme, modification status, charge state or the number of missed cleavages
- These groups are named based on the pattern below and are listed in the first column of the report followed by the modifications that are detected in this group and the peptide sequences

IGHG1_HUMAN Ig gMM-1;W264,N273,Q269,N267,N272

Protein Name

List of Amino Acid residues and protein positions