

# Mascot DIA enables direct comparison of DDA and DIA identification and quantitation results

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## 1. Background

Mascot DIA uses spectrum centric probabilistic scoring to match chimeric DIA MS/MS spectra without deconvolution of the spectra. The same sequence matching and precursor quantitation algorithms are used for DDA and DIA spectra, allowing results to be directly compared and the same downstream processing to be used between the two techniques.

## 2. Questions

- Do equivalent search settings give equivalent matches and scores?
- How does sequence and protein coverage compare between DDA and DIA processing?
- How does precursor quantitation coverage, accuracy and precision compare between DDA and DIA processing?

## 3. Methods

We reanalysed SCIEX Triple-TOF 6600 DDA and DIA raw files from PRIDE project PXD028735 (Puyvelde *et al.*, Scientific Data 9:126, 2022), using Mascot Distiller 3 beta and Mascot Server 3.2 beta in DDA and DIA modes.

Samples A and B were run in triplicate and combined. Results were rescored using Percolator with MS2PIP TTOF5600 (Declercq *et al.*, Nucleic Acids Research W1:51, 2023) and DeepLC3 full\_hc\_PXD005573\_pub (Bouwmeester *et al.*, Nature Communications 17:2601, 2026) models.

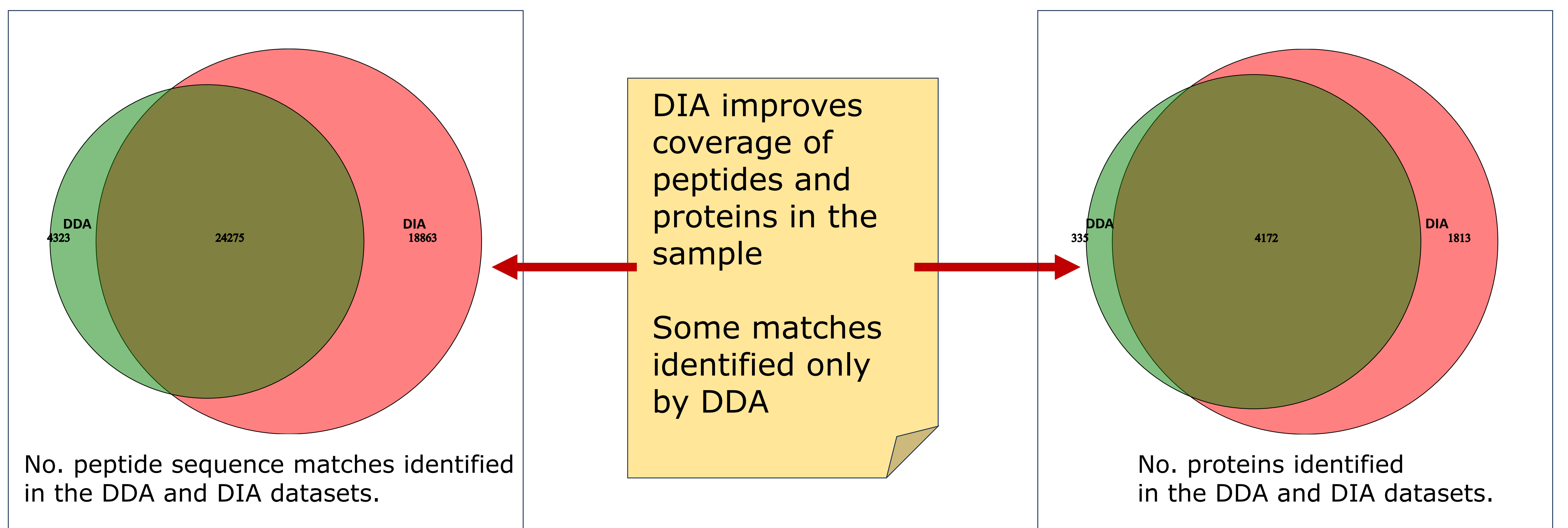
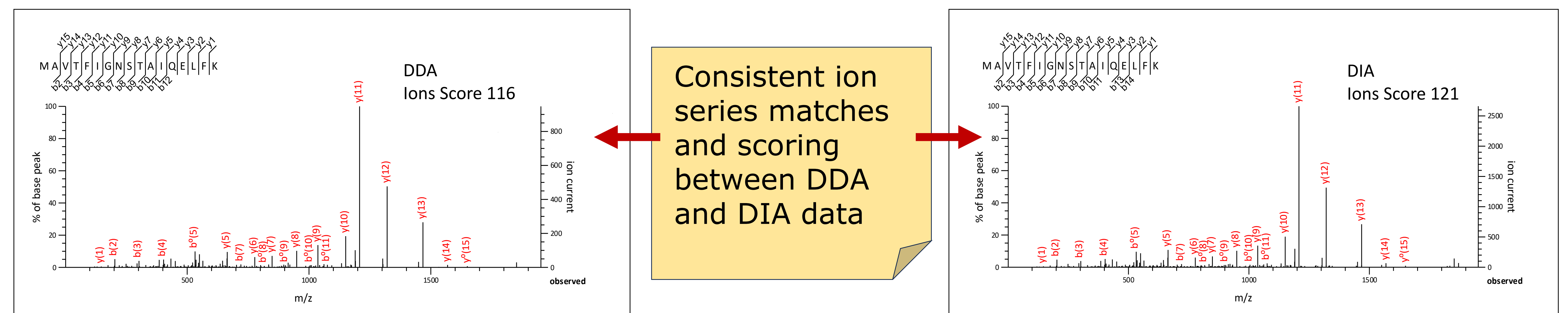
Precursor quantitation was carried out in Mascot Distiller. Human, yeast and *E. coli* proteins have expected log<sub>2</sub> protein ratios 0, 1 and -2.

*Accuracy: median log<sub>2</sub> peptide ratio per species*

*Precision: median absolute deviation (MAD) of the ratio*

COI: Ville Koskinen and Patrick Emery are directors and minority shareholders of Matrix Science Ltd.

## 4. Results and conclusions



	Expected	DDA			DIA		
		Median	Precision	n	Median	Precision	n
<b><i>H. sapiens</i></b>	0	0.001	0.15	17292	0.03	0.11	19745
<b><i>S. cerevisiae</i></b>	1	0.98	0.19	5424	0.93	0.2	6089
<b><i>E. coli</i></b>	-2	-1.82	0.58	826	-1.71	0.93	1097

Minor changes in accuracy and precision between DDA and DIA

~14% more peptides quantified in the DIA dataset after XIC quality thresholds applied