

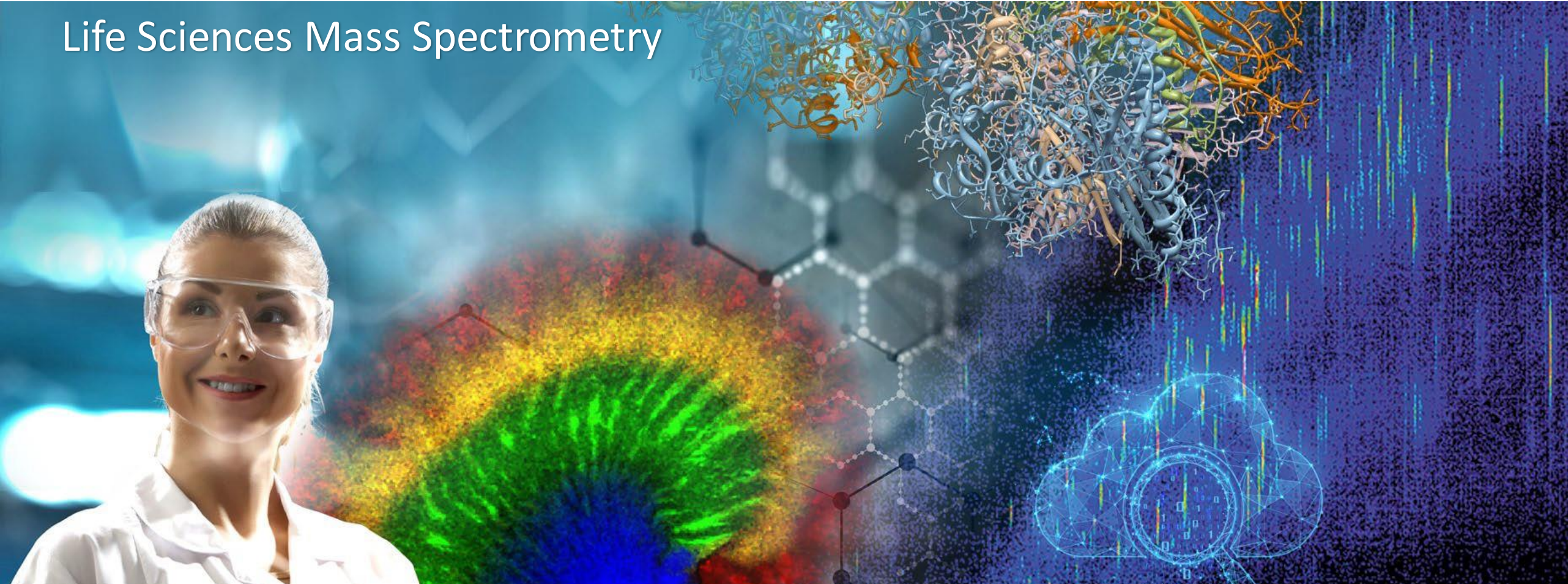
# High-precision ion mobility calibration for dia-PASEF analysis increases proteome coverage for high throughput 4D-Proteomics



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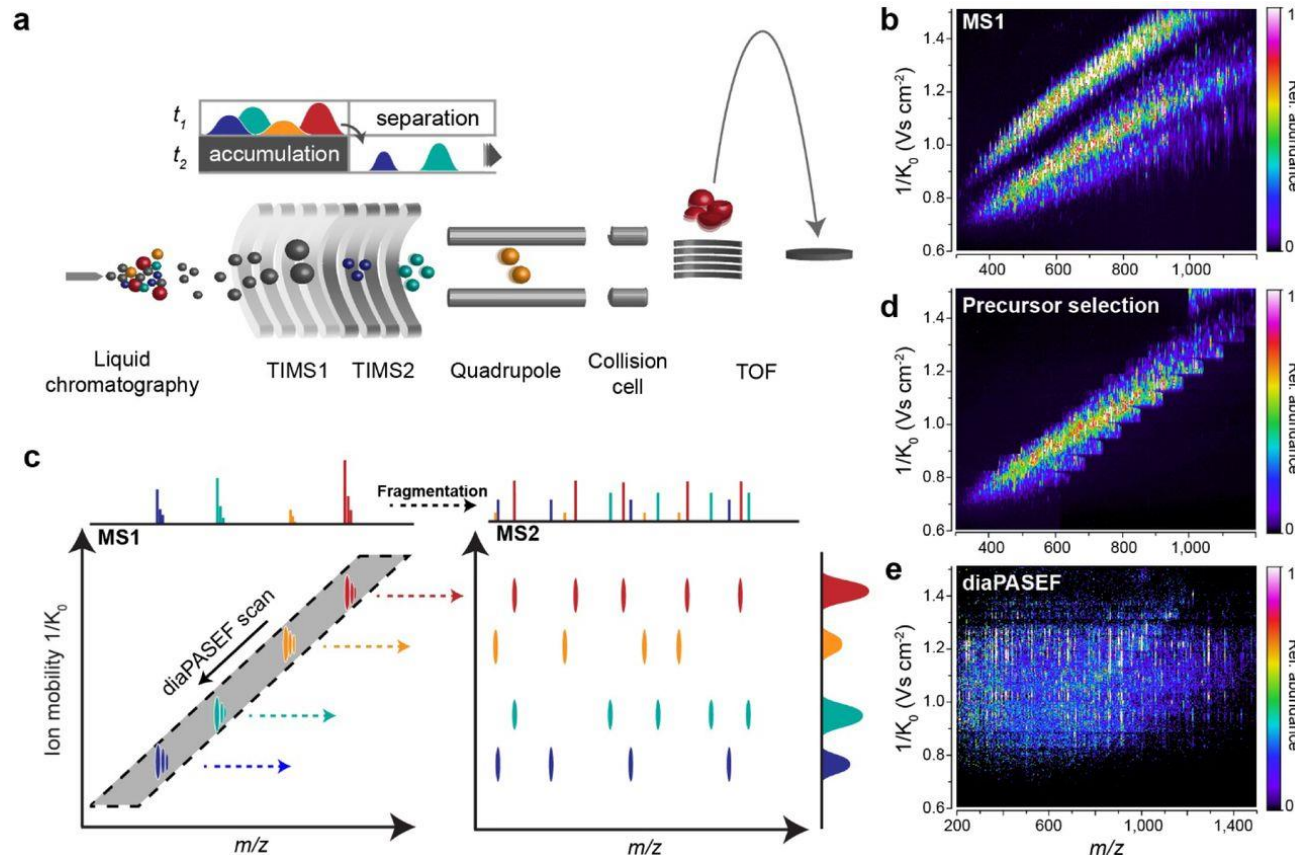
## Life Sciences Mass Spectrometry





# Introduction - dia-PASEF<sup>®</sup> + high precision IM calibration

**dia-PASEF** combines the well-known PASEF method, which utilizes trapped ion mobility spectrometry to achieve high duty cycle, efficient ion usage, extremely high sequencing speed, improved peptide identification rates and reproducibility of identification across multiple samples with DIA

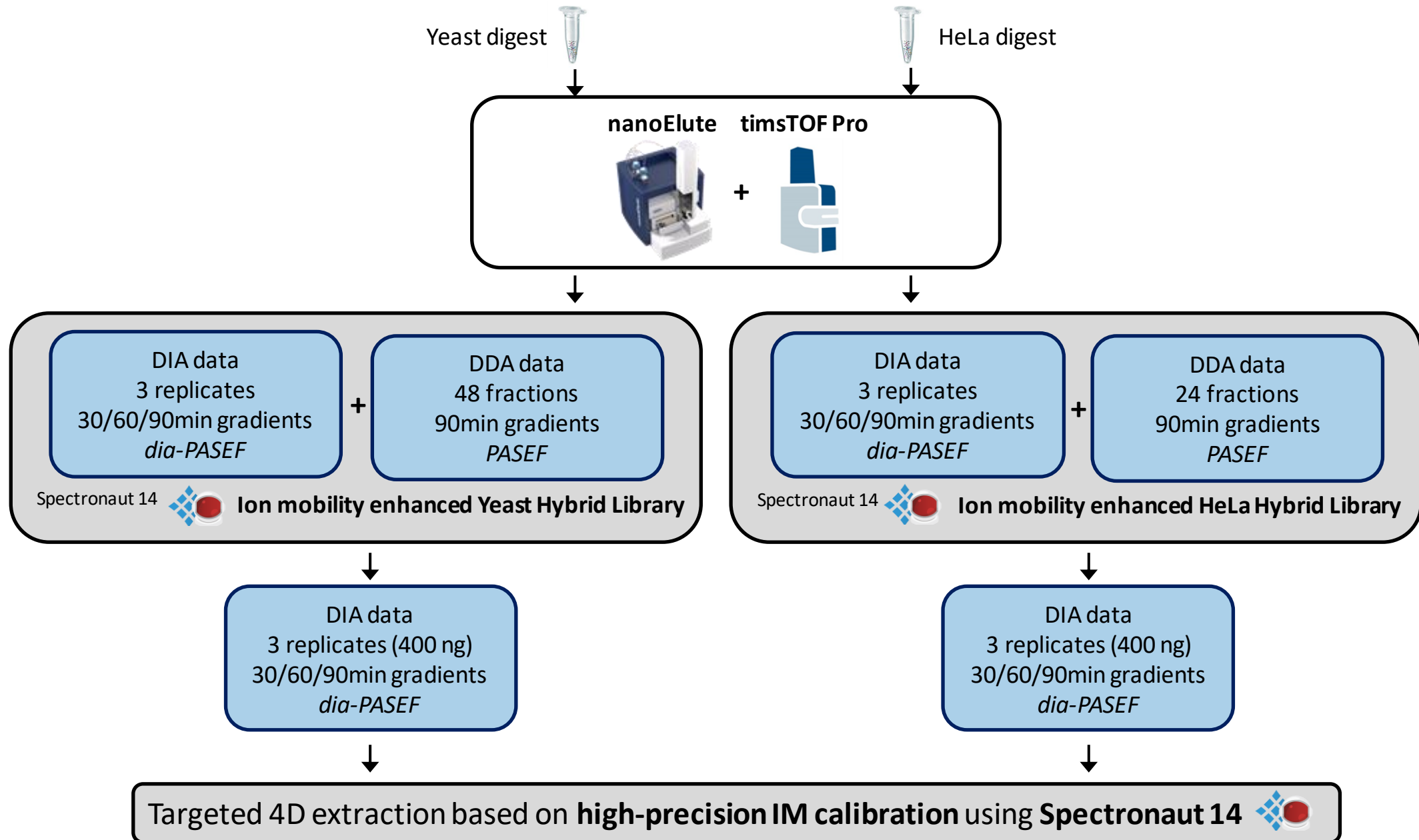


A novel **high-precision ion mobility (IM) calibration** workflow was designed in **Spectronaut 14** to improve ion mobility based DIA data analysis.

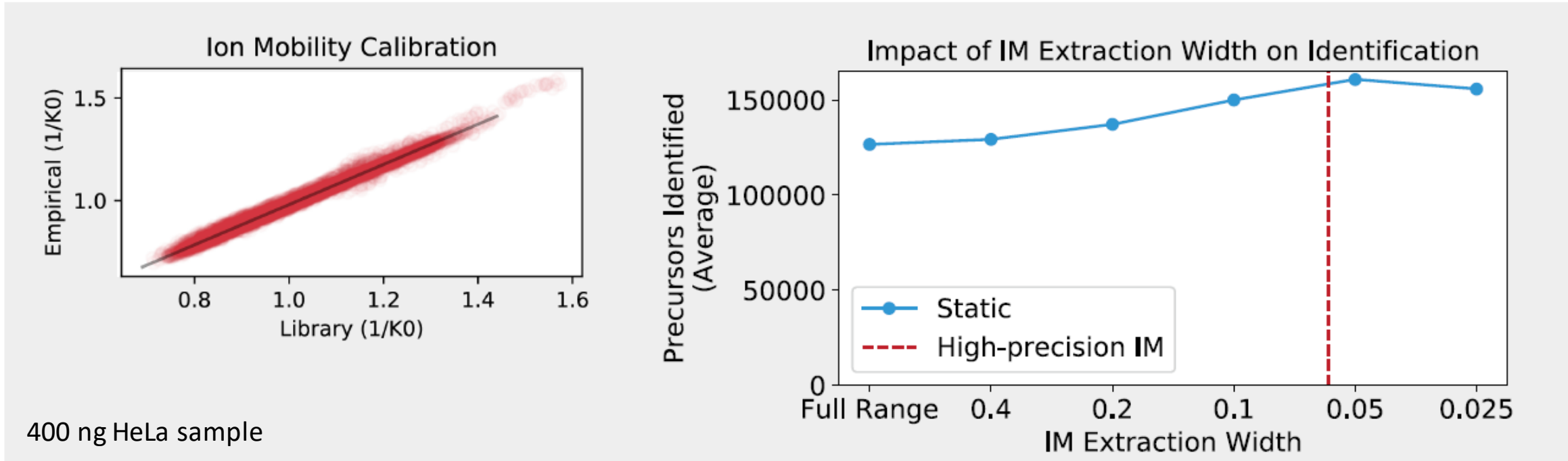


=> Here, we combine Bruker's dia-PASEF technology with this workflow to investigate its utility for complex proteomics samples using different gradient lengths

# Methods – Analysis workflow



# Methods – High precision ion mobility calibration in Spectronaut 14

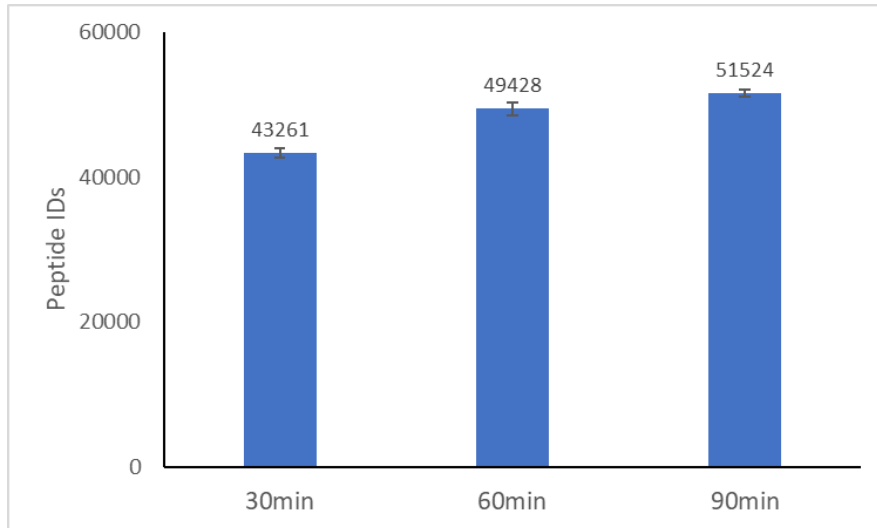


Ion mobility calibration ensures that optimal IM extraction width is automatically used for data analysis.

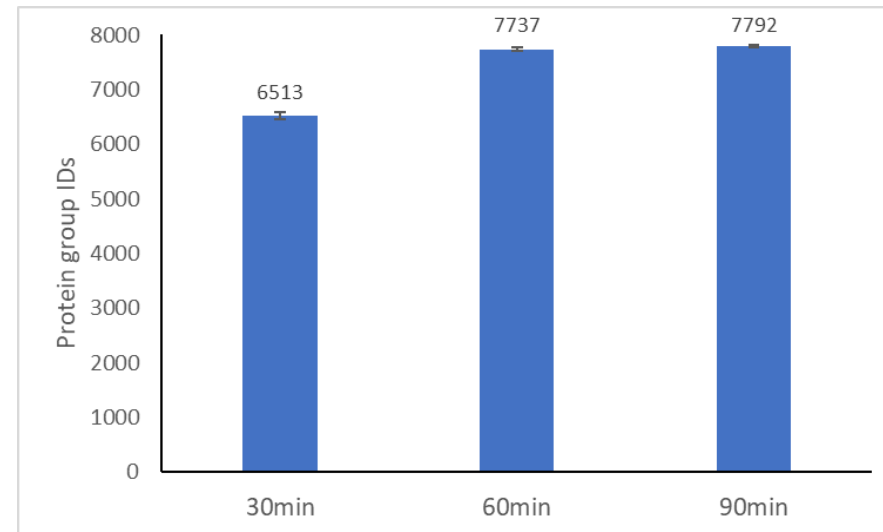
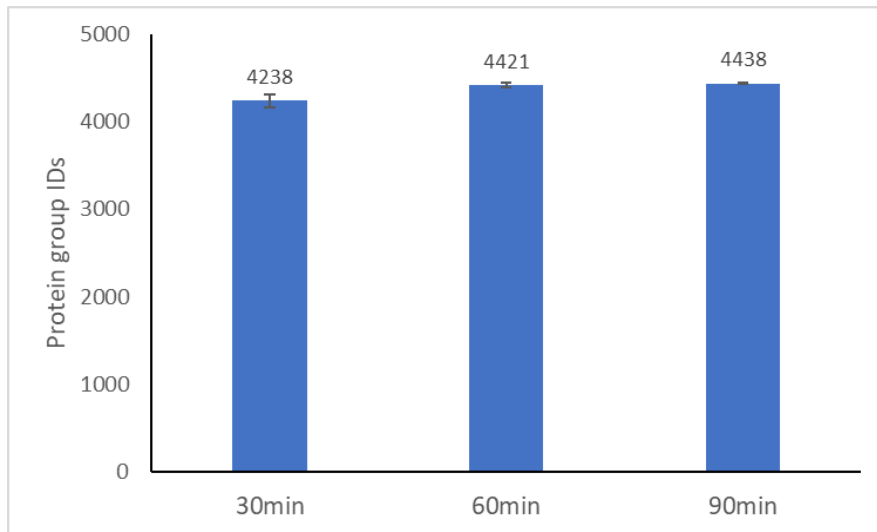
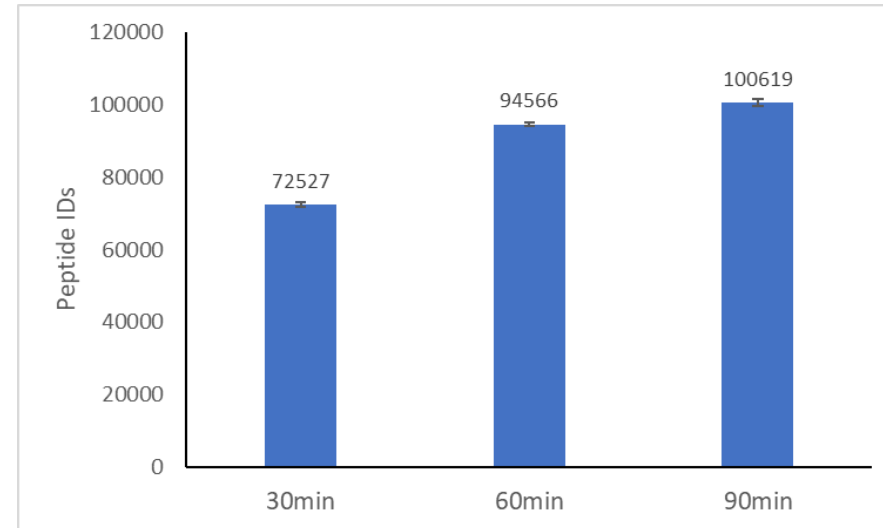
# Results – Increased identification rates using ion mobility calibration



## Yeast digest



## HeLa digest

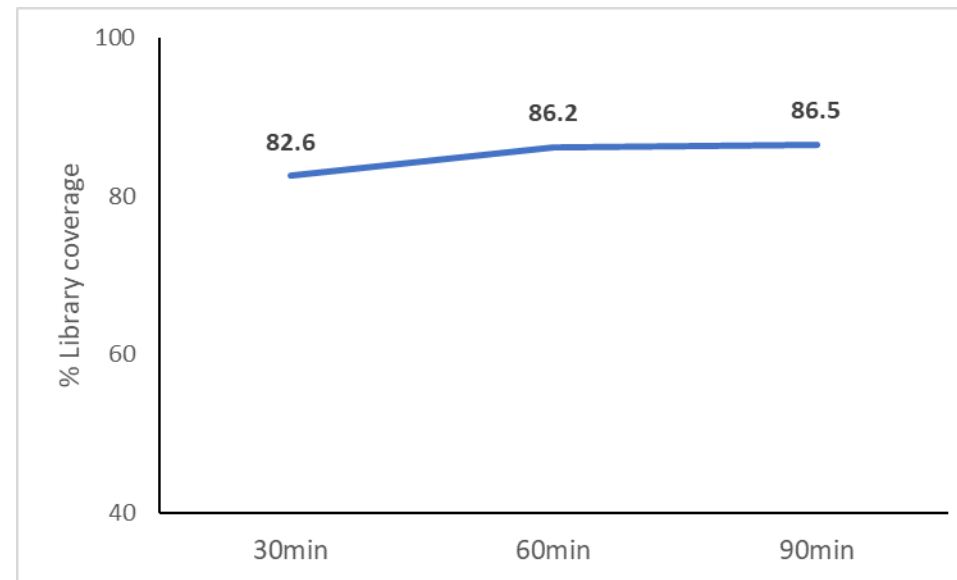


On average 4421 (Yeast) or 7737 (HeLa) protein groups identified from a single shot 60min gradient run.

# Results – Effect of gradient length on library coverage

<b>Hybrid Library</b>	<b>Yeast</b>
<b>Peptides</b>	87624
<b>Protein groups</b>	5129

Yeast digest



86.5 % library coverage in 90 min Yeast runs without fractionation.

The combination of shorter gradients with **high-precision ion mobility calibration dia-PASEF** analysis using Spectronaut 14 achieves **deep protein coverage** and is ideally suited for **high-throughput** proteomic profiling.

- dia-PASEF results in excellent results for different gradient lengths.
- Targeted 4-dimensional extraction based on high-precision IMS calibration allowed for the identification of more than 4200 Yeast protein groups in a single-shot 30 min gradient resulting in a near-complete proteome coverage.
- Excellent identification rates have been reproducibly achieved for complex HeLa digest with > 7700 protein groups identified at 90 min gradient time and > 6500 protein groups using a 30 min gradient.