Ion Mobility High Resolution QTOF MS - Impact of PASEF on detection of post-translational modifications

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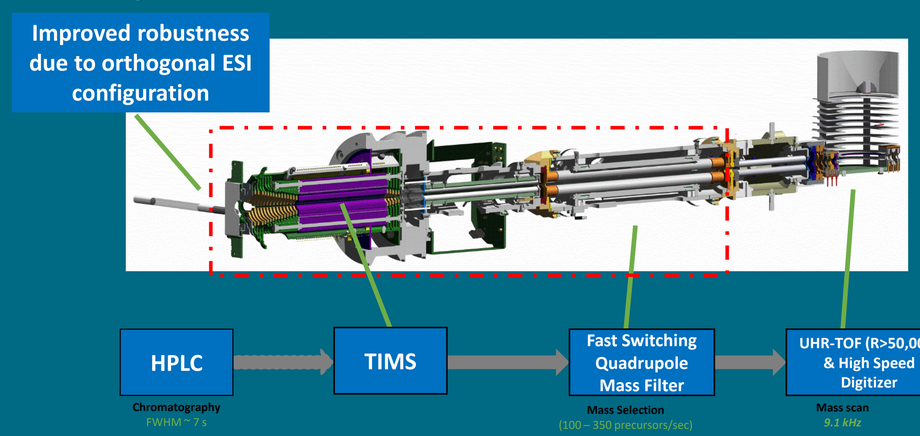
The field of molecular medicine is moving beyond genomics to proteomics. The goal being the characterization of the cellular circuitry and the understanding of the impact of disease and therapy on cellular networks.

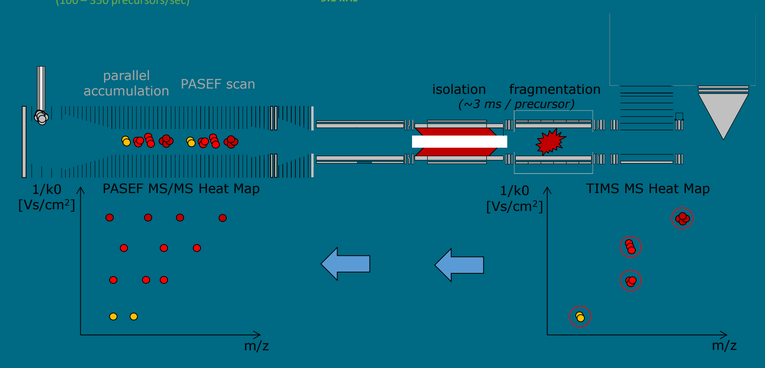
The impact of post-translational modifications in signal transduction and as triggers of autoimmune diseases is evident.

In this study we have evaluated the impact of chromatographic conditions and ability of PASEF to dig deeper into the PTMed proteome.

Schematics and ion-transfer system of the Bruker timsTOF PRO



Parallel Accumulation Serial Fragmentation (PASEF) acquisition allows ions to be accumulated in discrete ion packages at chromatographic speed and high duty cycle.



PTMs in Clinical Proteomics

Post-translational modifications (PTMs) of proteins are implicated all key biological processes. Signal transduction mechanisms and enzymatic modifications in autoimmune diseases generate dynamic and sub- stoichiometric levels of PTMs. Most strategies aimed at detecting PTMs necessitate efficient enrichment for deep coverage of the proteoforms, however, limits an unbiased detection of PTMs.

Multiple PTMs do not allow enrichment due to lact of methods.

From tissue extracts obtained from human, mouse and pig origin we isolated the proteome. Fractions of samples were separated with or without high pI fractionation (Thermo Sci) and analyzed without PTM enrichment by UPLC-PASEF-MS (Bruker timsTOF PRO using Ionoptiks 25cm Aurora nanoLC column) and other high-end MS. The proteome and protein modifications were characterized by database searching using BSI PEAKS PRO as well as MS-GF+ using high-performance supercomputing (HPC).

PSM vs PTMs

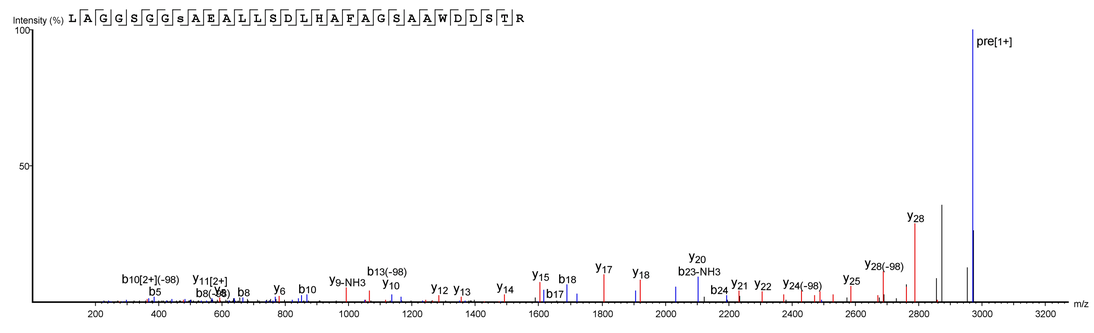
DDA vs PASEF based data acquisition

Enabled far higher number of PSM and percentage of PTMs assigned (data not shown).

(below) single phosphorylated tandem MS indicating clean, noiseless spectra of high coverage of fragment ion spectra

Peptide-Spectrum Matches	407136
Peptide sequences	107008
Protein groups	9619
Proteins	11900

Deamidation	.98	NQ	28367	101.38	2.08E4	20.75
Dehydration	-18.01	DSTY,C-term	6921	84.87	1.48E4	0.00
Acetylation	42.01	Protein N-term	4817	82.32	1.43E4	1000.00
Pyro-glu from Q	-17.03	N-term	4573	84.34	2.66E4	1000.00
Citrullination	.98	R	1650	73.25	7.94E2	1000.00
Formylation	27.99	K,N-term	1595	78.08	1.82E4	1000.00
Oxidation	15.99	DKNPRY	1232	72.54	3.91E4	15.91
Pyro-glu from E	-18.01	N-term	1139	81.31	5.51E3	1000.00

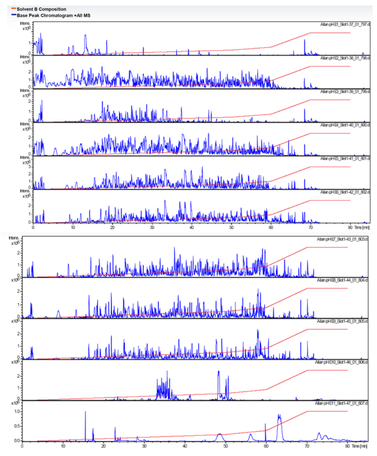


Protein fractionation

Extensive protein fractionation increases the final number of PSMs and detection level of individual PTMs.

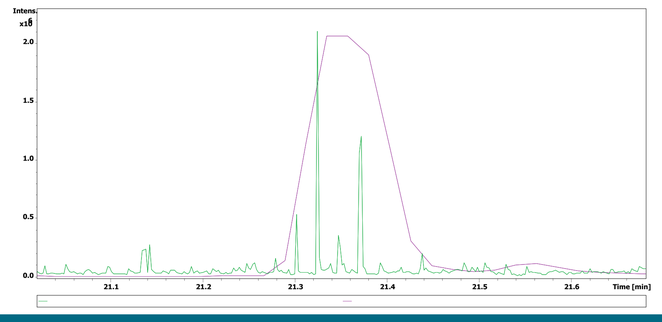
(right) UPLC-tandemMS of pI fractions.

(below) Protein group ID number (200ng on-column load) and XIC of single peptide



Protein groups	8546
Proteins	12715

pH E1	498
pH E2	5129
pH E3	3227
pH E4	5160
pH E5	5302
pH E6	5129
pH E7	5096
pH E8	4391
pH E9	4038
pH E10	1646
pH E11	48



Bioinformatics solutions

- The BSI PEAKS IMS allows easy for beginners and experts alike to analyze search results.

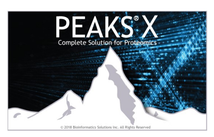
- PASEF tandem MS spectra of PTMs are clean and without much noise and CCS values to validate PTMs.

- HPC based database searching using MS-GF+ is feasible and fast (depending on number of nodes)

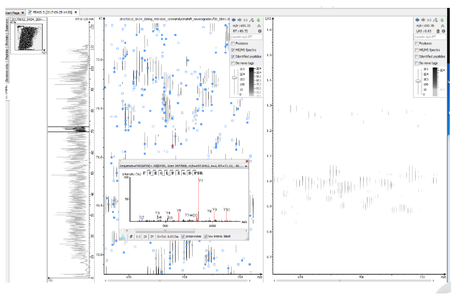
The BSI PEAKSX allows extraction of accurate CCS values (1/k0)

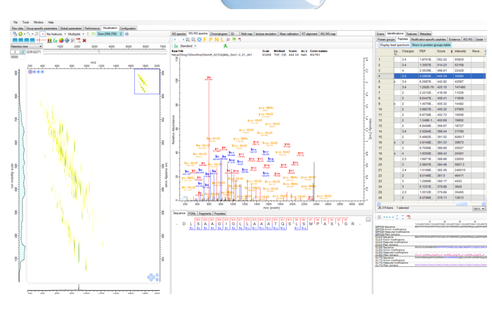
Mass	Length	ppm	m/z	RT	1/k0
3369.7490	33	0.0	1124.2570	61.31	1.0168-1.0407
2255.1003	18	0.0	752.7074	25.69	0.9085-0.9325
2240.0110	19	0.0	747.6776	43.16	0.9660-0.9899
2041.0367	18	0.0	1021.5256	71.97	1.1662-1.1901
2038.9669	19	0.0	1020.4907	57.21	1.1413-1.1652
2029.0579	19	0.0	1015.5362	47.47	1.1480-1.1719

Peaks studio



MaxQuant





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