Maximizing information content in ion mobility-enhanced DIA using overlapping, ion mobility-encoded quadrupole windows

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INTRODUCTION

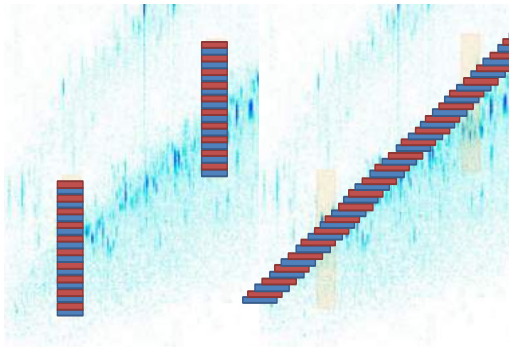
Cycling deterministically through segments of a predefined precursor m/z range, data-independent acquisition approaches provide a comprehensive record of all detectable precursor and fragment ions by isolating and fragmenting populations of different precursor ions. The recently introduced diaPASEF method utilizes the correlation of molecular weight and ion mobility in a trapped ion mobility device (timsTOF Pro 2) to extend sensitivity and specificity of established targeted data extraction workflows by the additional ion mobility dimension. Here, we discuss a novel DIA scan mode, which uses mobility-specific micro-encoding of overlapping quadrupole windows to maximize information content in DIA acquisitions.

METHODS

Total protein extracts from HeLa cells were digested and analyzed via nanoLC coupled to a timsTOF Pro 2. Spectral libraries were generated from triplicate PASEF-DDA acquisitions of high pH reversed-phase fractions of each sample. Overlapping ion-mobility-dependent quadrupole windows were defined based on spectral library data to provide >99% library coverage over the full mass range. Datasets were acquired on a modified timsTOF Pro 2 platform in both diaPASEF and several variations of the novel acquisition scheme (varying cycle time, quadrupole window size and overlap) with and without collision energy to evaluate and optimize acquisition workflow performance. Precursor-fragment deconvolution tools were trained on unfragmented HeLa datasets.

DATA ACQUISITION CONCEPT

diaPASEF mobility-scan DIA



RESULTS

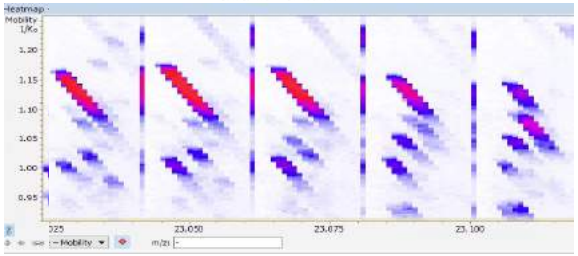


Figure 1: Mobility-scan DIA produces unique data patterns and increases sensitivity by overlapping diagonals.

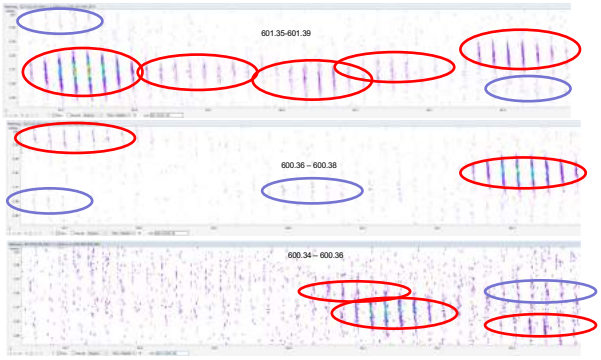


Figure 2: Mobility-scan DIA data complexity on fragment ion level
DIA analysis of 200 ng HeLa digest on a modified TimsTOF Pro2 platform.
Extracted ion chromatograms of the indicated mass ranges were generated in Data Analysis viewer. Discernible features are highlighted.

_ID	scan_325	scan_326	scan_327	scan_328	scan_329	scan_330	scan_331	scan_332	scan_333	scan_334
1	0	0	0	0	0	0	0	0	0	0
2	145	0	0	0	0	0	0	0	0	0
3	46	0	71	0	0	42	0	61	0	0
4	0	0	0	57	0	0	0	0	0	52
5	0	70	0	0	0	0	0	60	0	0
6	0	0	0	0	0	0	0	90	0	55
7	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0

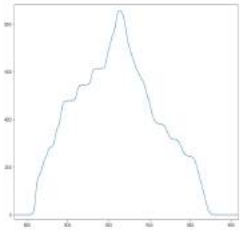


Figure 3: Low-intensity mobility-scan DIA data pattern enable precursor position modelling
Algorithmic prediction of the most likely precursor ion position based on a data occupancy matrix enables to pinpoint precursor ion positions with an accuracy of a few Da.

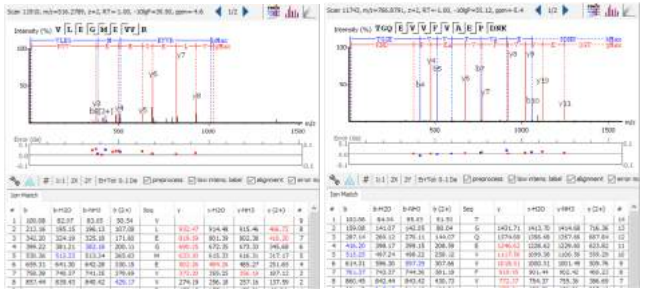


Figure 4: Database search results of deconvoluted mobility-scan DIA data
Deconvoluted mobility-scan DIA data from a HeLa digest were exported as .mgf files and submitted to Mascot Search. Exemplary peptide identifications are shown.

	DDA1	DDA2	DDA3	DDA4	DDA5	DDA6	DDA7	DDA8	MSDIA	count DDA
b3	2402.9	7336.4	419.76	448.28	255.36	270.52	28.001	482.28	12726	8
b4	802.82	4029.7	488.96	316.39	39.195	28.042	73.934	184.24	5090	8
b6-NH3	393.58	1401.8	137.57	218.76	129.03	66.787	104.03	119.07	2378	8
y10	39485	136530	5478.9	15052.6	4288.3	3257.9	106.64	3533.3	121628	8
y3	2857.4	11412	345.2	994.8	271.39	265.26	223.04	146.6	10179	8
y4	2014.8	5093.6	120.26	349.87	132.03	68.979	50.714	37.041	9202	8
y6	3936.4	10116	399.31	1519.63	175.72	300.07	131.93	48.535	15109	8
y7	2533.6	10035	228.47	638.9	224.72	185.66	48.246	35.355	11959	8
y8	4452.8	15756	429.13	1066.8	532.38	405.54	91.537	201.98	21666	8
b2	2093.4	7269.4	488.36		113.22	49.757	33.963	207.79		7
y5	1634.9	7101.5	317.82	522.32	176.15	52.91		79.635	11836	7
y9	7213.9	26118	622.52	2620.7	904.96	1010.3		1298.5	39181	7
b6	578.22	3414.4	258.247	34.824			19.367	58.056	3541	6
b7	882.74	2798.1	219.534	283.45	58.56			154.66	2559	6
y10-NH3	5574.7	16047		372.06	755.15	419.4	118.41		11893	6
y8-NH3	498.55	1702.4		57.324	20.401	26.124	19.266		2238	6
b5	915.36	3124.3	297.7	263.26			33.599		2919	5
b8-NH3	669.57	2509.9	68.134	73.081				72.791	2458	5
b5-NH3			106.6	111.64		73.777		42.717		4
b8-H2O	168.01	1250.3		10.573	51.151				2832	4
b9	956.08	2221	92.37	259.89					3119	4
b9-H2O	239.98	2098.6	128.61	96.742					2732	4
b9-NH3	487.51	712.93		4.5617		39.956			2880	4
y10-H2O		1354.9	165.72	100.85				39.452	5083	4
y11	5961.5	18546		1534.65		530.13			6400	4
y11[2+]			17.314		117.68		136.37	169.37	13444	4

Figure 5: Exemplary fragment ion coverage provided by mobility-scan DIA compared to eight reference DDA runs.

CONCLUSIONS

- Novel quadrupole scanmode provides the basis for comprehensive, fast and highly sensitive DIA
- Using overlapping quadrupole selection windows, the method can theoretically provide a 2.5-fold increase in fragment ion sensitivity compared to diaPASEF
- To enable in-depth data processing of resulting high-complexity datasets, we developed algorithms for multidimensional peak detection and to classify whether the intensity distribution of a given fragment matches the one of a target precursor to generate highly specific pseudo-MSMS spectra on the basis of multidimensional deconvolution (RT, IMS, quad window), which can be converted into common MS/ MS data formats such as mgf and searched directly with established tools like PEAKS and Mascot

ACKNOWLEDGEMENTS

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