In Depth Analysis of Host Cell Proteins from Antibody Preparations using PASEF

Introduction

In the rapidly expanding arena of biotherapeutic analysis and bioprocess development, the analysis of host cell proteins (HCPs) at the ppm level is critical. ELISA is currently the gold standard for QC applications but non-targeted methods such as mass spectrometry are now emerging - the advantages of mass spectrometry include rapid method development and identification of all detected proteins. The low abundance of HCPs still presents a significant challenge and initial work in this field has often required specialized setups. In this work we show how PASEF (parallel accumulation and serial fragmentation) scans, as implemented on the timsTOF PRO QTOF, can be applied to HCP analysis using routine analytical- or nanoflow configurations to

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achieve the goal of sensitive detection with enhanced speed and data quality.

Methods

The NISTmAb Reference Material 8671 and the Universal Proteomics Standard (UPS1, Sigma) were separately reduced using DTT in TFE solution and alkylated with iodacetamide prior to overnight digestion with trypsin (Promega). Peptides were separated on an Intensity Solo 2 1.8 µm C18 100 x 2.1 mm column using an Elute UHPLC coupled to a timsTOF Pro ion mobility QTOF mass spectrometer (all-

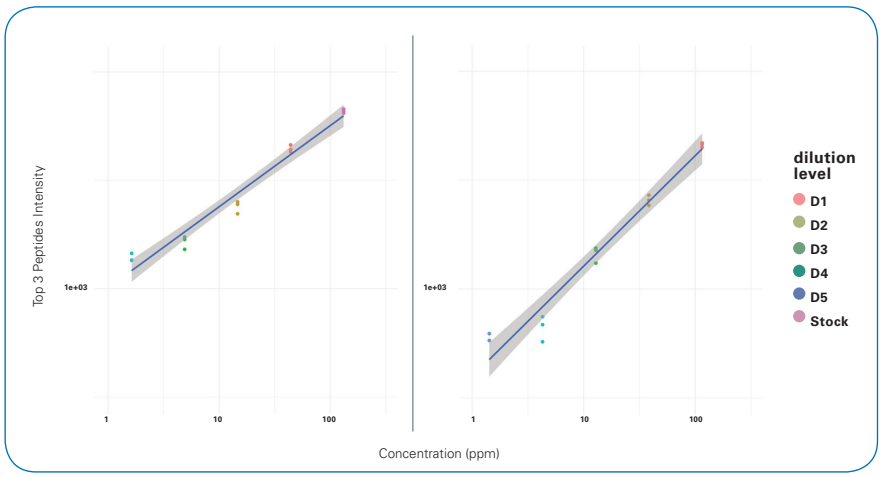


Figure 1: Quantitation of beta-2-microglobulin and NAD(P)H dehydrogenase Top 3 quantitation was performed using PEAKS Studio software (BSi)

Protein	#Peptides	Seq. Cov. [%]	Scores	MW [kDa]	pI
Fructose-bisphosphate aldolase A	20	64,3	902.6	39,3	8,3
Fructose-bisphosphate aldolase C	13	47,1	679.4	39,4	6,7
Glucose-6-phosphate isomerase	10	24,6	322.5	62,7	8,1
Protein disulfide-isomerase A6	5	13,4	222.3	48,1	5,0
Granulins	4	12,1	103.6	63,4	6,4
Beta-2-microglobulin	3	22,7	116.8	13,8	8,6
Low affinity immunoglobulin gamma Fc region receptor II	3	10,0	75.0	36,7	6,2
Adenylate kinase 2, mitochondrial	3	17,2	65.8	26,5	7,0

Figure 2: HCPs identified in NISTmAb by analytical 1D-UHPLC-MS/MS

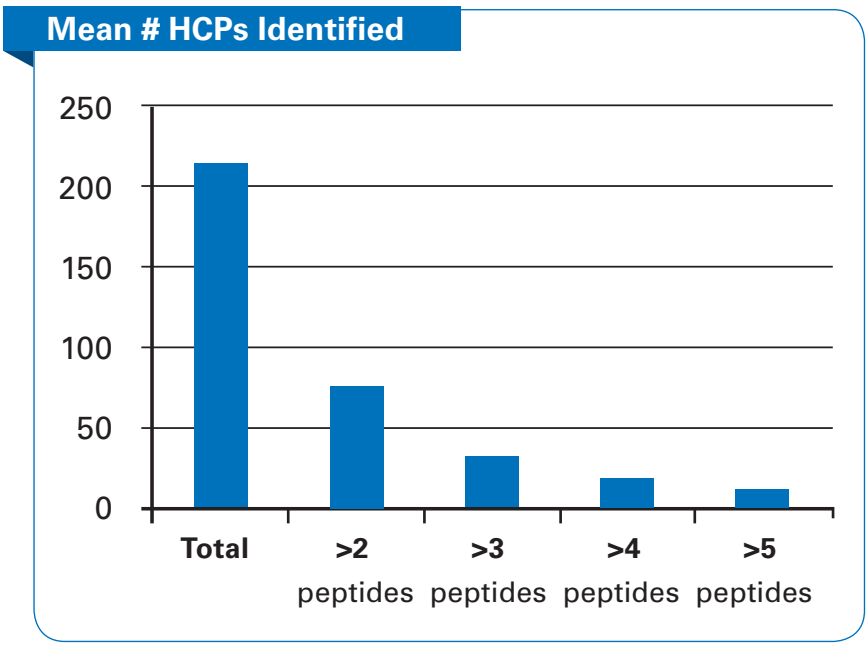


Figure 3: The number of HCPs identified in the NIST mAb using nanoflow UHPLC (1% FDR, Ion score > 20, n=2).

Bruker Daltonics). A 150 minute gradient was used in a total runtime of 165 minutes. For nanospray, a nanoElute UHPLC was fitted with an IonOpticks 25 cm x 75 µm 1.6 µm C18 column and a 210 minute gradient was used. PASEF scans were recorded and the spectra searched against a mouse SwissProt database using the Mascot Search Engine (Ion Score 20, 1% FDR).

Results and Discussion

The UPS1 standard was used to make a 5-step 1:3 dilution series in a constant background of NISTmAb over a concentration range from 0.3 to 934 ppm. Each dilution was measured in triplicate and the amount of NISTmAb

loaded on column was 25 µg for each injection. PASEF enabled the detection of UPS1 proteins down to low single digit ppm concentrations in the presence of 25 µg NISTmAb. Figure 1 shows the linear response for two of the UPS1 proteins, beta-2-microglobulin and NADPH dehydrogenase, illustrating the suitability of this approach for HCP work.

The experimental setup described above was also used for the measurement of the actual HCPs in the NISTmAb sample. In this instance 30 µg NISTmAb was loaded onto the column and resulted in the detection of 8 proteins with 3 or more peptides and a further 10 proteins with 2 peptides, at 1 % FDR (Figure 2). For example, Glucose-6-phosphate isomerase and Beta-2-microglobulin were identified with 9 and 3 unique peptides respectively. These results demonstrate the use of PASEF to identify HCPs using a routine 1D-UPLC-MS configuration.

Protein	# Peptides	Seq. Cov. [%]	Scores	MW [kDa]	pI
Fructose-bisphosphate aldolase A ***a**	32	76,9	2065,1	39,3	8,3
Fructose-bisphosphate aldolase C	19	64,7	1479,7	39,4	6,7
Glucose-6-phosphate isomerase	19	50,4	1060,1	62,7	8,1
Granulins *	14	42,8	662,6	63,4	6,4
Papilin	11	12,7	448,7	138,8	8,1
Hepatocyte growth factor-like protein	9	16,2	378,9	80,6	7,7
Polypeptide N-acetylgalactosaminyltransferase 6	8	20,6	338,1	71,5	8,8
Peptidyl-prolyl cis-trans isomerase FKBP2	7	48,6	277,8	15,3	9,1
Protein disulfide-isomerase A6	6	12,7	406,3	48,1	5,0
Syntaxin-12	5	27,0	300,2	31,2	5,3
Adenylate kinase 2, mitochondrial	5	33,1	253,8	26,5	7,0
Heterogeneous nuclear ribonucleoproteins A2/B1	5	15,9	227,9	37,4	9,0
NSFL1 cofactor p47	5	20,0	210,3	40,7	5,0
Titin *	5	0,2	98,5	3904,1	5,9
Clathrin interactor 1	4	6,7	218,1	68,5	5,9
Adenylyl cyclase-associated protein 1	4	16,2	193,9	51,5	7,2
Beta-2-microglobulin	4	16,0	192,5	13,8	8,6
Eukaryotic translation initiation factor 4B	4	9,5	142,9	68,8	5,5
Ubiquitin-conjugating enzyme E2 variant 2	4	37,9	135,7	16,4	7,8
Protein NipSnap homolog 3B *	4	25,1	92,2	28,3	9,5
Heterogeneous nuclear ribonucleoprotein A1 ***b**	3	12,8	201,7	34,2	9,3
Methionine--tRNA ligase, cytoplasmic	3	6,0	192,6	101,4	6,8
Semaphorin-4B	3	3,4	142,3	91,3	8,5
Fumarate hydratase, mitochondrial *	3	12,0	133,3	54,3	9,1
Stress-induced-phosphoprotein 1	3	6,1	133	62,5	6,4
Heterogeneous nuclear ribonucleoprotein A/B	3	16,1	122,5	30,8	7,7
Properdin	3	9,5	114,2	50,3	8,3
Protein ABHD11	3	21,5	109,7	33,5	9,6
Nucleoprotein TPR *	3	2,1	108,8	273,8	5,0
Protein enabled homolog	3	4,4	105,2	85,8	7,6
Ubiquitin-60S ribosomal protein L40 *	3	29,7	102	14,7	9,9
40S ribosomal protein S12 ***c**	3	21,2	87,1	14,5	6,8
Dapper homolog 3 *	3	1,8	74,8	63,2	10,4
Polypeptide N-acetylgalactosaminyltransferase 2	3	5,1	73,7	64,5	8,8
Bcl-2-associated transcription factor 1 *	3	3,8	71,1	105,9	10,0
Pancreatic lipase-related protein 2 *	3	13,1	60,6	54,0	6,2

*Table 1: Abridged list of HCPs detected in NISTmAb with 3 or more peptides using nanoflow UHPLC. Example MS/MS spectra shown in Figure 4 for HCPs marked a,b,c. *HCPs previously unreported for NISTmAb*

In instances that require deeper coverage of HCPs, mAb samples can also be analysed using a standard proteomics setup using nano UHPLC. The timsTOF Pro powered by PASEF has already been established as the new benchmark for bottom-up proteomics applications ([1], [2]). In comparison to other nano LC setups, robustness is improved by the CaptiveSpray ion source, which allows ions to be sprayed directly from the emitter for increased sensitivity. Figure 3 shows that > 200 HCPs were identified in 1.5 µg NISTmAb, including 78 proteins which were identified with 2 or more peptides. The identified proteins include the expected NISTmAb HCPs and many previously unreported HCPs (Table 1).

The identification of HCPs on this scale is possible because Trapped Ion Mobility Spectrometry (TIMS) separates ions based on collisional cross section using 2 TIMS cells in series. This additional dimension of separation makes it possible to focus precursor ions in a small window in time and space, which are selected with a specific m/z and collisional cross section using the PASEF scan mode (Figure 5). With PASEF, the timsTOF Pro features high sequencing speeds of > 100 Hz, whilst generating high quality MS/MS spectra that enable sensitive detection of low abundant HCPs, as exemplified in Figure 4.

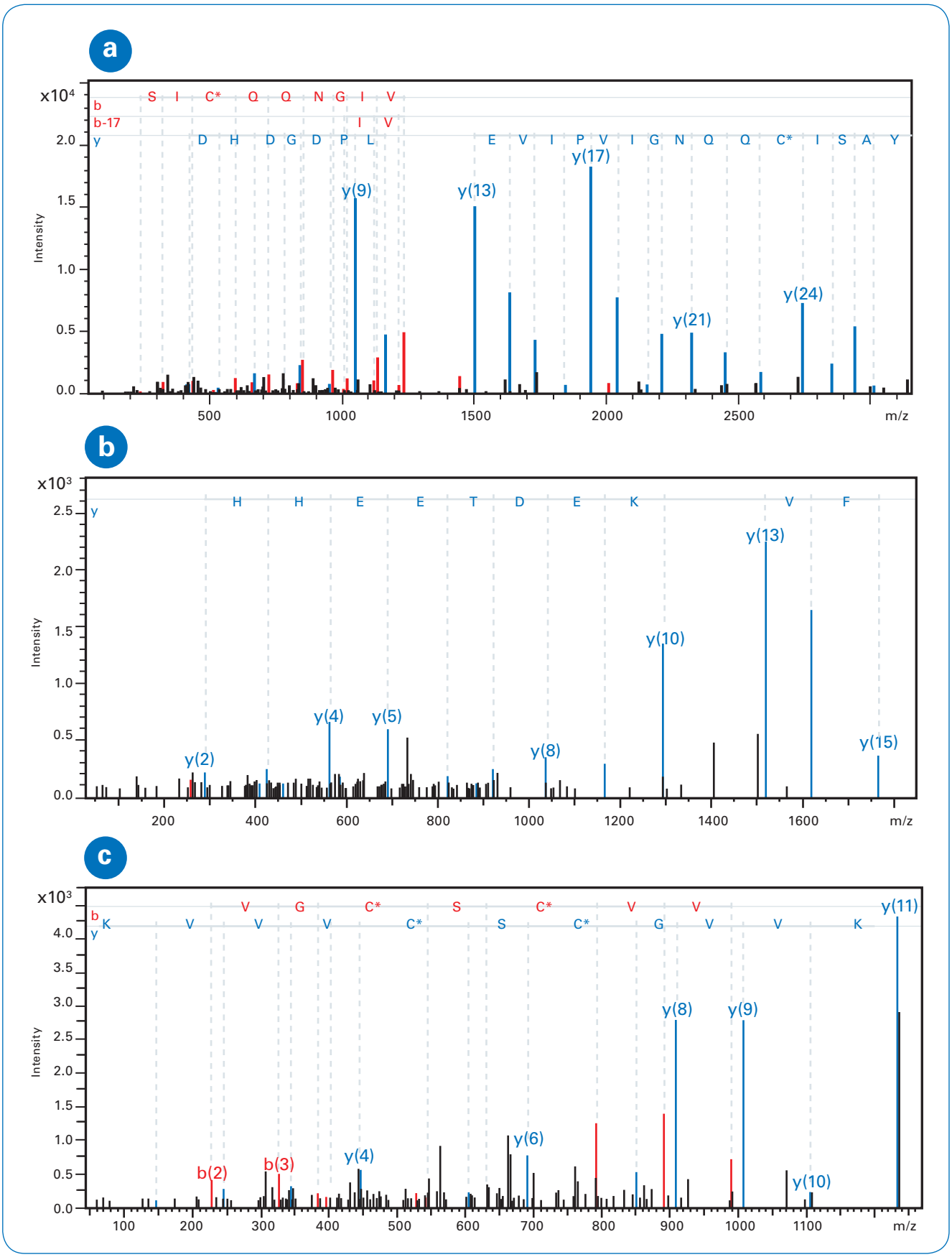
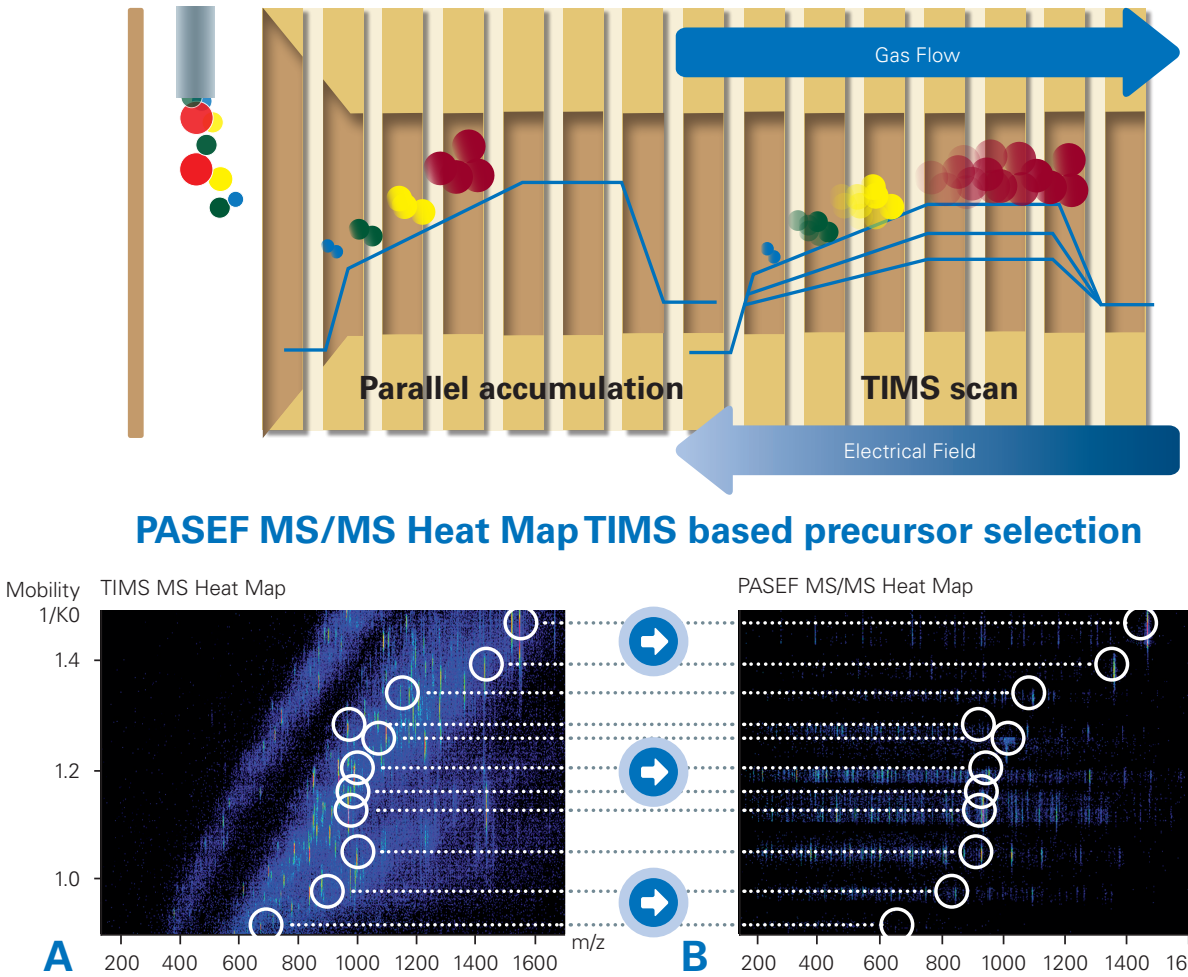


Figure 4: MS/MS spectra of a) Fructose-bisphosphate aldolase A b) Heterogeneous nuclear ribonucleoprotein A1 c) 40S ribosomal protein S12

Figure 5: Accumulation and focusing of precursor ions in the TIMS cell. Bottom: PASEF scan allows 2 dimensional precursor isolation



Conclusions

- PASEF scans improve the sensitivity of routine peptide mapping enabling the detection of HCPs at required sub 100 ppm levels
- PASEF coupled to nanoLC facilitates detection of previously unreported trace level HCPs
- The quality of MS/MS sequence spectra provided by the timsTOF Pro with PASEF allows high confidence in protein ID even with only 1-2 peptides
- The depth of HCP identification provided by PASEF technology allows fingerprinting of biomanufacturing processes and ability to easily identify the effects of changes in these procedures

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References
[1] Meier et al.; J Proteome Res. 2015 Dec 4;14(12):5378-87
[2] Lubeck et al.; Bruker Application Note LCMS 131, PASEF™ on timsTOF Pro, 09 / 2017

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