Advancing mAb Characterization with Microchip CE-MS Couples to a PASEF Enabled QTOF

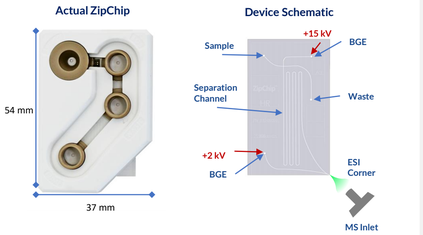
Scott Mellors[1], Guillaume Tremintin[2], Scott B. Ficarro[3], and Jarrod A. Marto[3]

[1]908 Devices, Inc., Boston, MA 02210; [2]Bruker Scientific, San Jose CA; [3]Departments of Cancer Biology and Oncology Pathology, Blais Proteomics Center, Dana-Farber Cancer Institute, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

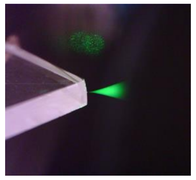
Introduction

Microchip CE-MS has recently emerged as a powerful tool for biotherapeutic characterization, achieving fast and efficient separations of analytes ranging from single amino acids or peptides, all the way up to fully native proteins and protein complexes. As innovation in MS technology continues to produce faster and more powerful instruments, microchip CE-MS applications benefit greatly. Here we take advantage of the new PASEF scan mode (Parallel Accumulation-Serial Fragmentation), which makes it possible to run faster separations without sacrificing the information content of the MS data. In this work we exploit this capability to demonstrate rapid and efficient characterization of a monoclonal antibody.

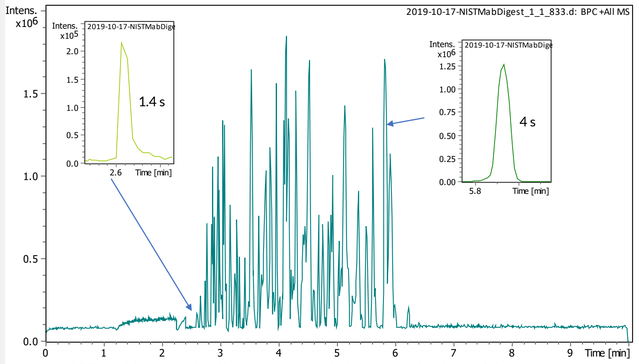
Methods



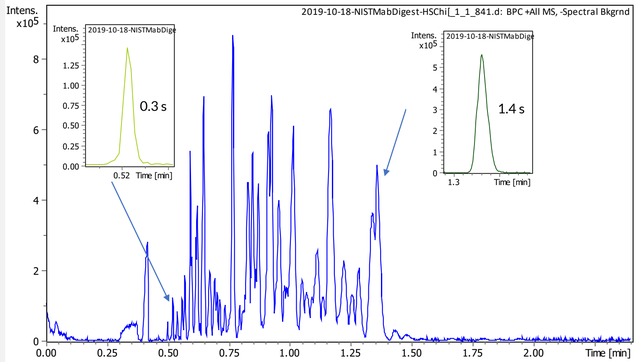
All work was performed with a microfluidic CE-MS system (ZipChip, 908 Devices) coupled to a TIMS enabled QTOF (timsTOF Pro, Bruker). The NIST mAb (SRM8671, NIST) was analyzed at both the intact and peptide level. The intact analysis was performed at pH 5.6 using the ZipChip Charge Variant TOF background electrolyte (BGE) and a chip with a 22 cm long separation channel (ZipChip HRN, 908 Devices). MS data were acquired with optimized settings from 1000-8000 m/z. The peptide mapping was performed at pH 2.3 using the ZipChip Peptides BGE. Two different chip types with different separation channel lengths were used: HR (22 cm) and HS (10 cm). The eluted peptides were detected using an optimized PASEF method.



Fast Peptide Mapping



NIST mAb tryptic digest run on High Resolution chip



NIST mAb tryptic digest run on High Speed chip

Coverage map for NIST mAb tryptic digest run on HR chip

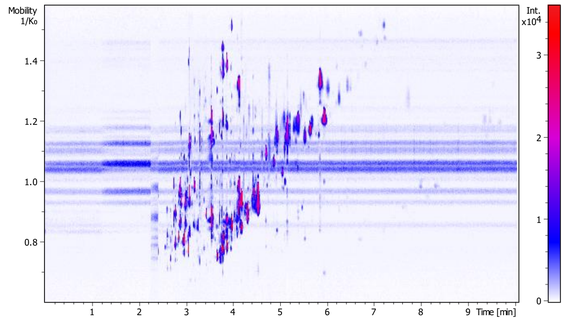
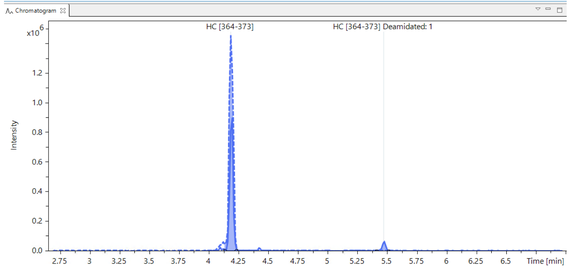


Image plot of Ion mobility versus microchip CE run time

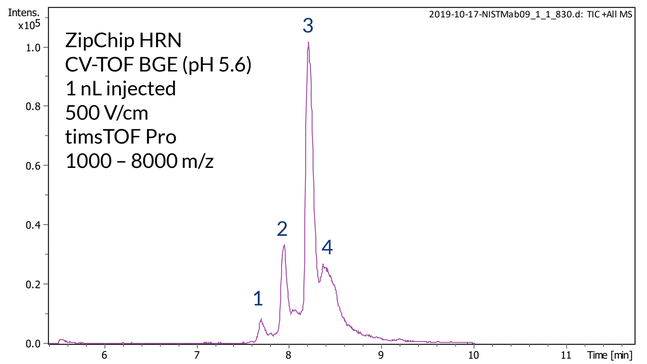
Row	Peptide	Range	Variable Mods	Sequence	z States	Mr Ref	Rel. Area [%]	Rt Ref [min]
1	HC 36	364-373	Deamidated: 1	NQVSLTCLVK	2	1161.6064	1.4	5.47
2	LC 11	126-141	Deamidated: 11	SGTASVVCLLNNFYPR	2	1797.8720	1.4	5.09
3	HC 23	278-291	Deamidated: 12	FNWYVDGVEVHNAK	2,3	1677.7787	2.8	3.80
4	LC 11	126-141	Deamidated: 12	SGTASVVCLLNNFYPR	2	1797.8720	1.4	5.09
5	LC 13,14	145-168	Deamidated: 13	VQWKVDNALQSGNSQESVTEQDSK	3	2677.2467	0.8	4.60
6	HC 26	305-320	Deamidated: 14	VVSVLTVLHQDWLNGK	2,3	1807.9832	15.6	3.72
7	HC 37	374-395	Deamidated: 14	GFYPSDIAVEWESNGQPENNYK	2	2544.1081	18.3	6.00
8	HC 37	374-395	Deamidated: 14, 19	GFYPSDIAVEWESNGQPENNYK	2	2545.0921	0.1	6.15
9	HC 37	374-395	Deamidated: 14, 20	GFYPSDIAVEWESNGQPENNYK	2	2545.0921	0.1	6.15

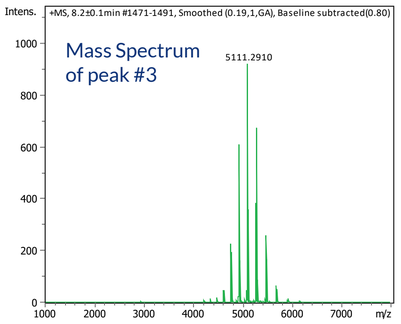


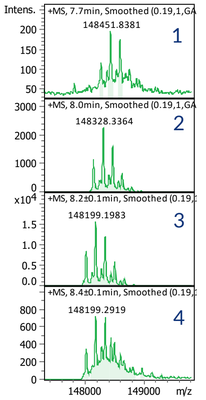
Separation of a deamidated peptide from its unmodified variant

A tryptic digest of the NIST mAb was first analyzed on a high resolution chip (ZipChip HR), using a PASEF MS/MS method. The full run time for this method was 10 minutes, with most of the peptide peaks eluting between 2.5 and 6 minutes, and having peak widths at half height ranging from 1.4 to 4 seconds. A base peak electropherogram of this separation is shown in the top left, and an image plot of the ZipChip migration time versus the ion mobility shown just to the right of it. The image plot shows how the combined power of liquid and gas phase separation is able to resolve a very large number of peptide species. Processing this data yields excellent sequence coverage as shown in the coverage map at left. This fast peptide mapping method can achieve resolution of modified peptides, as illustrated by the separation of a deamidated peptide above. With some fine tuning of the PASEF method, we believe we can achieve similar results with an even faster method using a high speed chip (ZipChip HS). An example of that separation is shown at middle-left, yielding only slightly lower resolution of species with a total run time about 4x faster.

Native Charge Variant Analysis







ZipChip Analysis of intact mAbs under native separation conditions yields separation of charge variants and high quality mass spec data. Here we demonstrate accurate identification of the primary charge variants (C-terminal lysines and deamidation) and glycoforms with excellent mass accuracy on the timsTOF pro. The deconvoluted spectra of the 4 main charge variant peaks and a table with identifications of the 3 most abundant glycans for each variant are both shown at right.

Peak #	Charge Variants	Glycans	Measured	Theoretical	Mass error	ppm
1	2K	G0F/G0F	148291.9	148293.5	-1.6	-10.5
		G0F/G1F	148451.8	148455.6	-3.8	-25.3
		G1F/G1F	148615.0	148617.5	-2.5	-16.8
2	1K	G0F/G0F	148163.7	148165.3	-1.6	-10.5
		G0F/G1F	148328.3	148327.4	0.9	6.3
		G1F/G1F	148490.2	148489.4	0.8	5.3
3	0K	G0F/G0F	148037.1	148037.1	0.0	0.3
		G0F/G1F	148199.2	148199.3	-0.1	-0.7
		G1F/G1F	148363.1	148361.2	1.9	12.5
4	0K1D	G0F/G0F	148038.0	148038.1	-0.1	-0.4
		G0F/G1F	148199.3	148200.3	-1.0	-6.8
		G1F/G1F	148364.3	148362.2	2.1	14.0

Conclusions

ZipChip separations coupled with a timsTOF Pro QTOF is a powerful platform for the characterization of biotherapeutic proteins. The high speed of the PASEF MS/MS data acquisition enables deep sequence coverage from fast ZipChip separations. Use of the high resolution native (HRN) chip and the charge variant-TOF (CV-TOF) BGE yields good sensitivity and accurate mass assignments for characterization of intact charge variants.