ZipChip CE-ESI-MS for Fast and Efficient Biopharmaceutical Characterization

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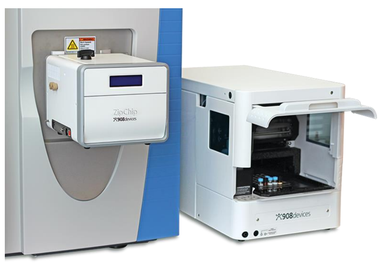
Overview

ZipChips are glass microfluidic devices that integrate a CE separation with electrospray ionization performed directly off of the corner of the device. Microfluidic integration of components enables extremely fast and efficient separations, while also making the system small, simple and easy to use. The ZipChip system has previously been available only on Thermo mass spectrometers, but we have recently completed development of a ZipChip interface that is compatible with Sciex mass specs. This presentation provides a demonstration of the capabilities of the ZipChip system when used in combination with a Sciex 6600 TripleTOF for applications relevant to biopharmaceutical labs.

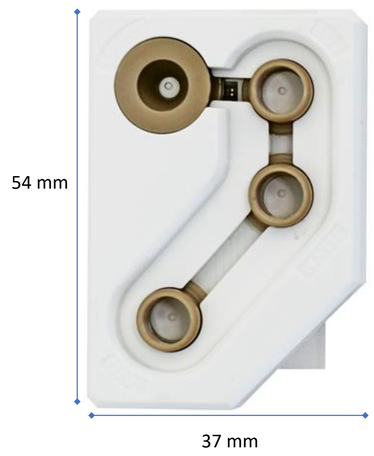


Methods

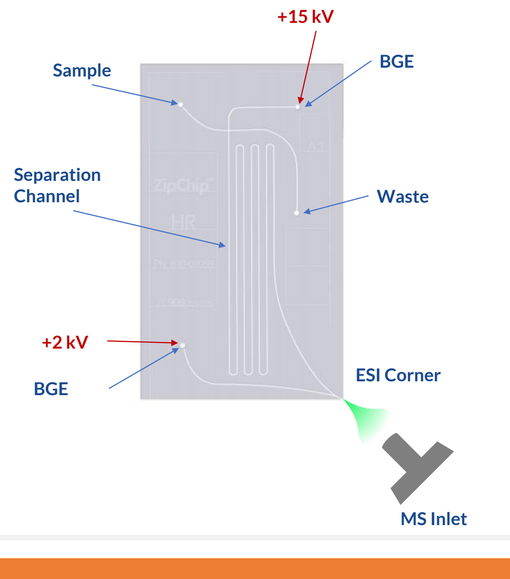
Instrumentation: All of the data on this poster was acquired using the new ZipChip Sciex Interface (ZC-SI, 908 Devices) mounted on a Sciex 6600 tripleTOF mass spectrometer. ZipChips are available with two different channel lengths; HS chips have a 10-cm long separation channel, while HR chips have a 22-cm long separation channel. All of the work demonstrated here utilized HR chips. All ZipChips utilize a covalently attached, neutral polymer surface coating to prevent analyte interactions and suppress electroosmotic flow. The charge variant work shown here, used a "high resolution native" (HRN) chip. This chip uses a new surface coating process to achieve high resolution protein separations under native conditions.

Sample Prep: Intact mAbs were diluted with background electrolyte (BGE) directly from their formulations to a concentration of 0.5 mg/mL. The NIST mAb subunit sample was digested with the IdeS enzyme and reduced with 100 mM DTT to yield Fc, Fd, and light chain subunits. The peptide mapping sample was digested with Trypsin using the Thermo Smart digest protocol.

Actual ZipChip

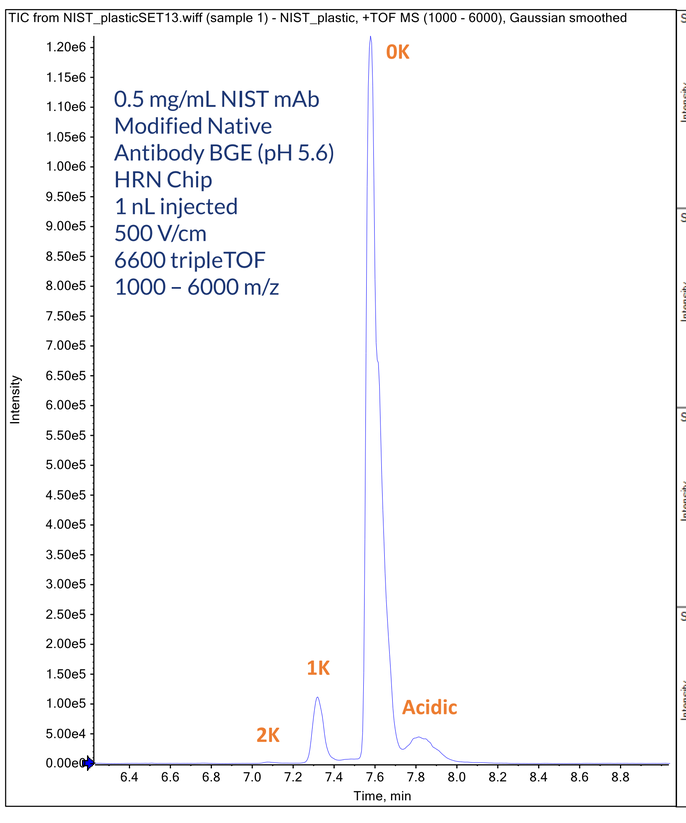


Device Schematic

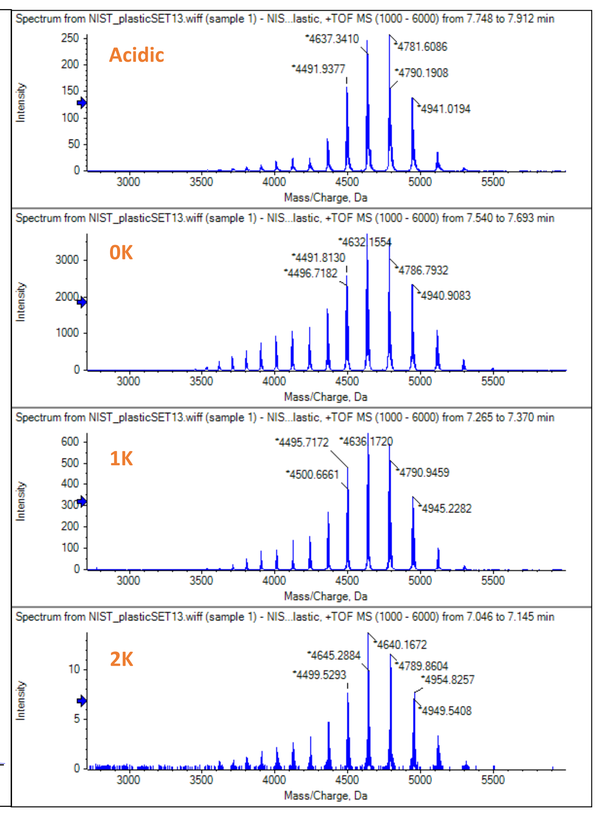


Native Antibody Charge Variant Analysis with ZipChip CE-ESI-MS

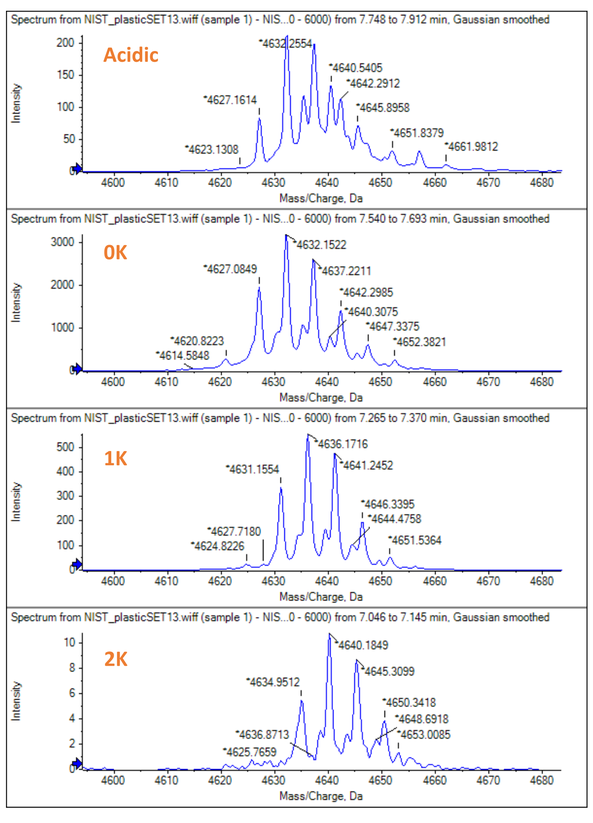
Electropherogram



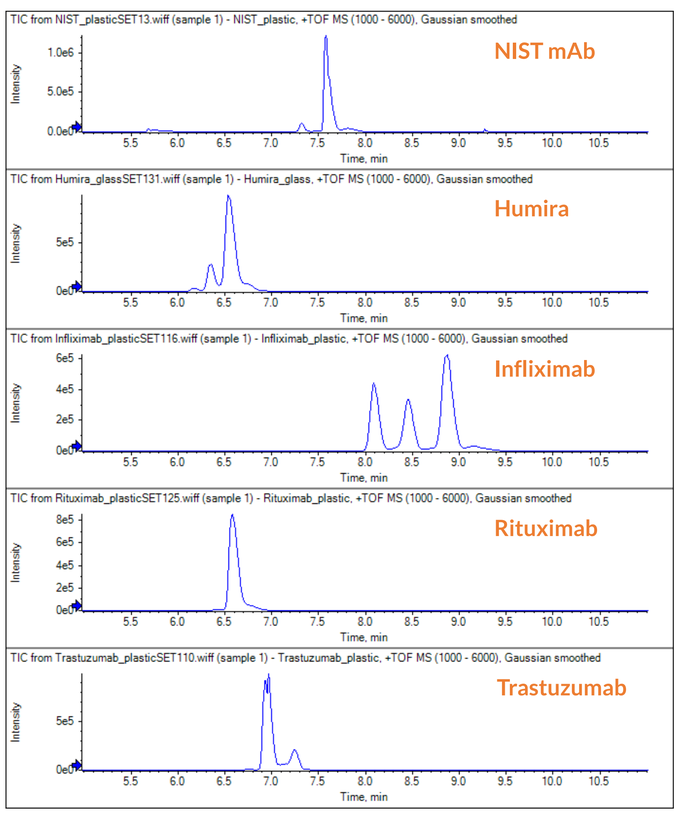
Full Spectra



+32 Charge State Zoom

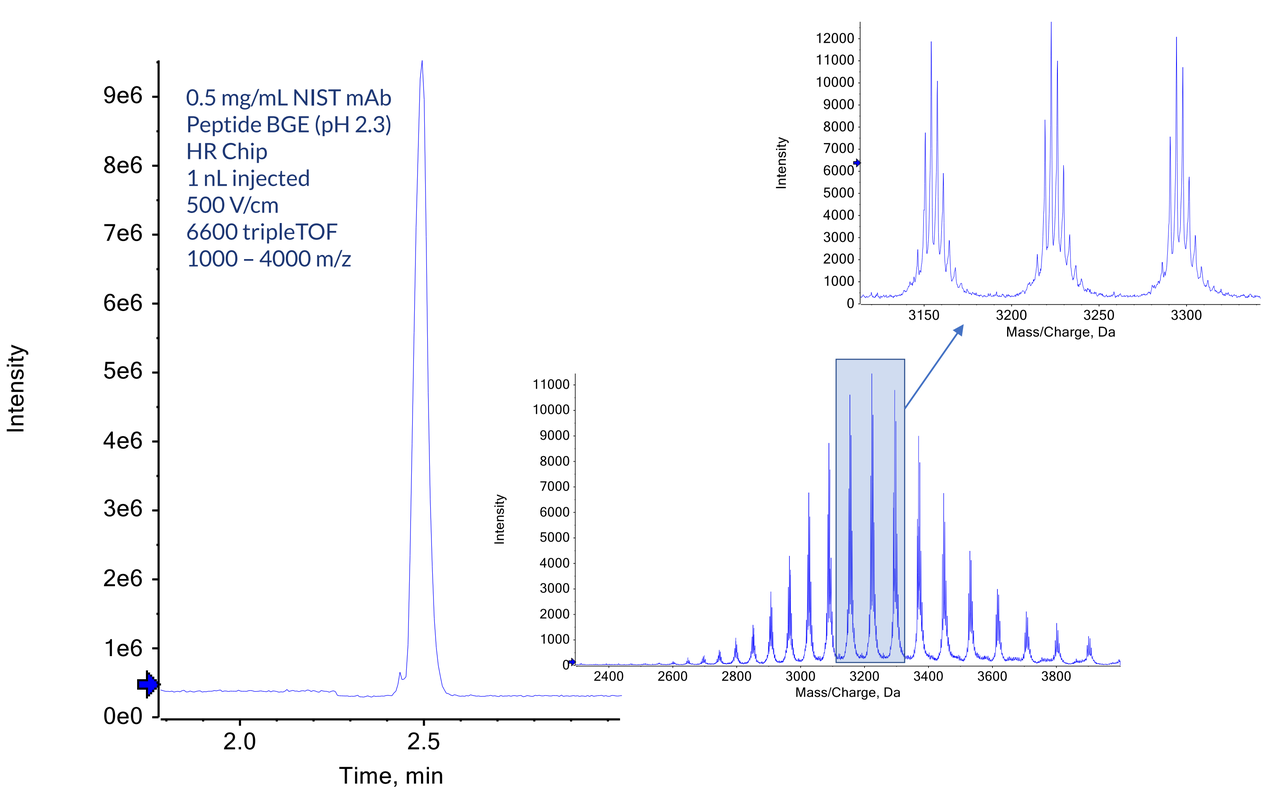


1 method for all mAbs

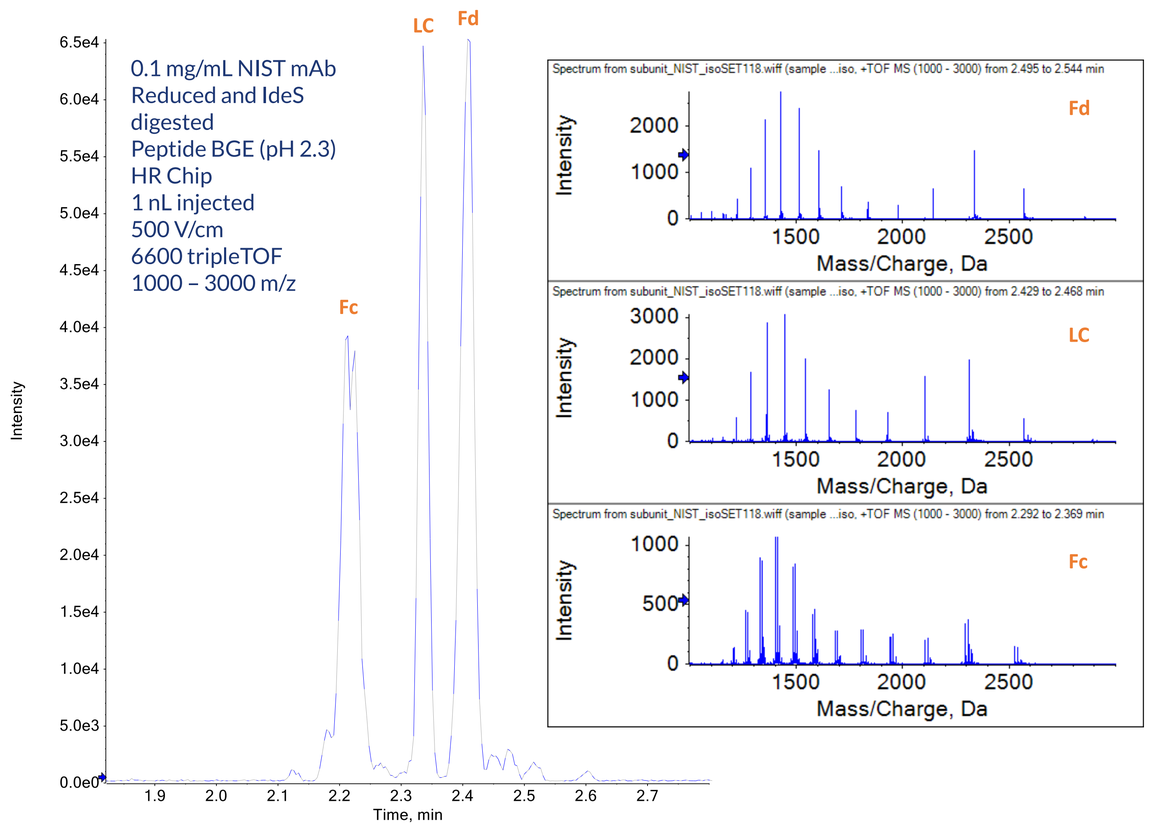


Multilevel Characterization

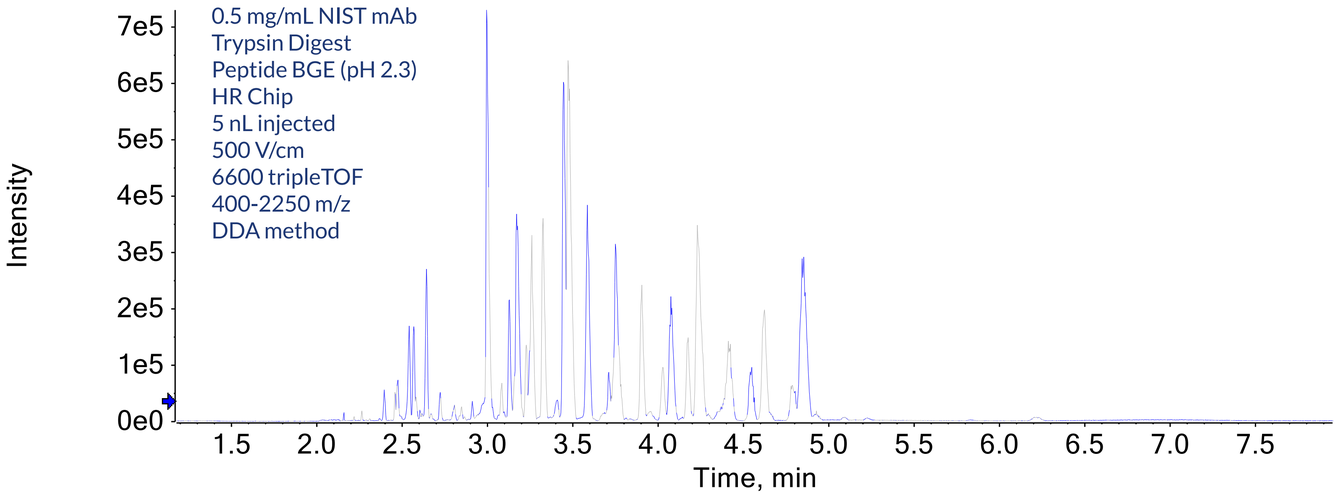
Intact Denatured Antibody



Antibody Subunits



Peptide Mapping



Summary and Acknowledgments

The work shown here demonstrates that the ZipChip methods developed for biopharmaceutical characterization can achieve excellent results when paired with the Sciex 6600 TripleTOF. The charge variant separation of the NIST mAb achieved baseline resolution and clear MS identification of the basic C-terminal lysine variants and deamidated acidic variants. The same method applied to 4 other biotherapeutic mAbs yielded excellent charge variant separations which closely match the well known charge variant characteristics of these molecules. The multilevel characterization performed in denaturing conditions demonstrates the versatility of methods that can be performed on the ZipChip system.

We thank Fang Wang and Zuzana Demianova of Sciex for hosting us in their lab, helping us to collect the data shown here, and providing the Humira, Infliximab, Rituximab, and Trastuzumab samples.