Native Analysis of Monoclonal Antibodies by Microchip Capillary Electrophoresis-ESI-MS

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Overview

The characterization of intact monoclonal antibodies by mass spectrometry is challenging due to the size and complexity of the molecules. The ability to separate charge variants in a separation that is directly coupled to the mass spectrometer greatly improves the MS analysis by minimizing spectral overlap of similar variants, while also providing a charge variant separation which aids in identification of the species. We have previously demonstrated the ability to do such an analysis using microfluidic capillary electrophoresis – ESI-MS under "near native conditions". This current work extends that capability by operating under fully native conditions which maintain the folded structure of the molecules both during the CE separation and through the ESI transition into the gas phase.

Methods

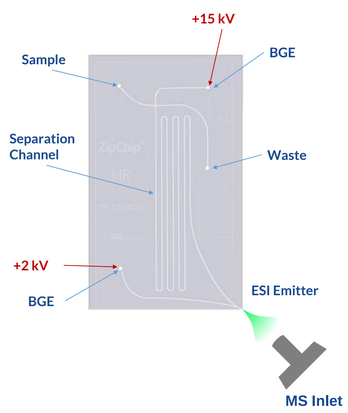
All work was performed using a commercially available microfluidic CE-ESI system (ZipChip, 908 Devices Inc.), attached to an orbitrap mass spectrometer (Exactive EMR or Q Exactive HF-X Biopharma, ThermoFisher). The microfluidic devices utilized a covalently attached, neutral polymer surface coating to prevent analyte interactions and suppress electroosmotic flow. An optimal background electrolyte (BGE) was developed to maintain fully native conditions while maximizing the resolving power of the separation and the sensitivity of the ESI-MS. The method was initially tested with the NIST monoclonal antibody reference material (RM 8671, purchased from NIST), along with approved drug molecules trastuzumab, and trastuzumab emtansine (T-DM1) which were kindly provided by collaborators. All samples were diluted directly from formulation to a concentration of 1 mg/mL. No other sample prep was done. All samples were run with the same separation method. Data were processed using Biopharmafinder 1.0 (ThermoFisher).

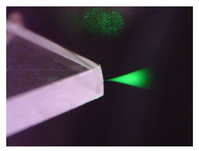
ZipChip Method:
- High resolution chip (HR, 22 cm separation channel)
- 1 nL injection volume
- 500 V/cm
- Pressure assist enabled at 0.5 minutes
- 10 minute analysis time

Exactive EMR Mass Spec Method:
- EMR mode (HCD pressure 6)
- SID 150/CE 60
- S Lens RF: 100
- Inlet temperature 200 C
- 3 microscans

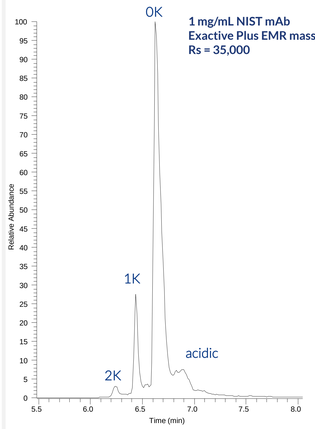
QE HF-X Biopharma Mass Spec Method:
- HMR mode (HCD pressure 1.5)
- SID 150
- Funnel RF: 100
- Inlet temperature 200 C
- 3 microscans

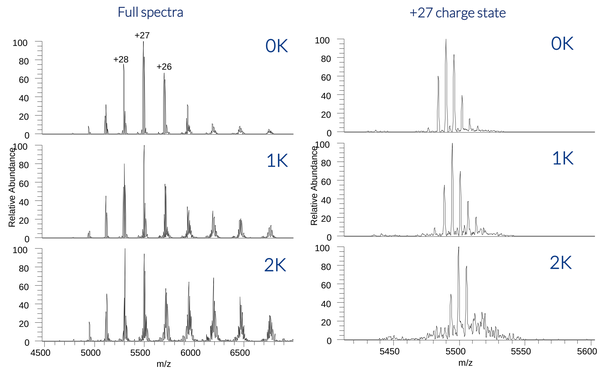
Device Schematic

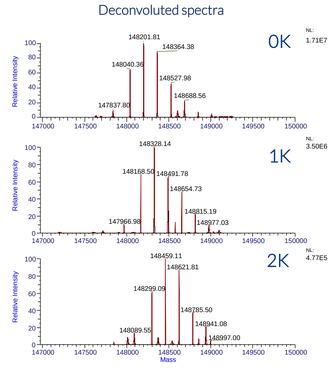


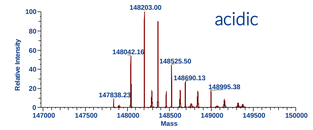


Native Analysis of NIST mAb



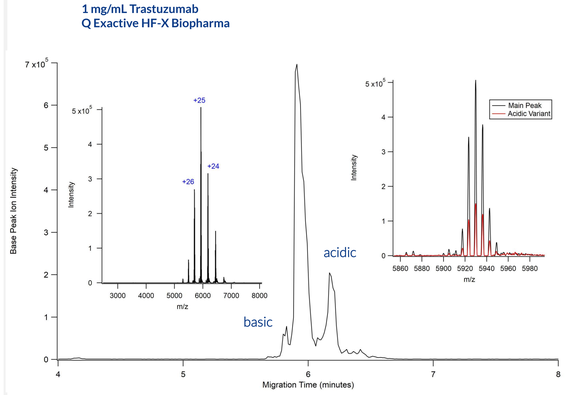


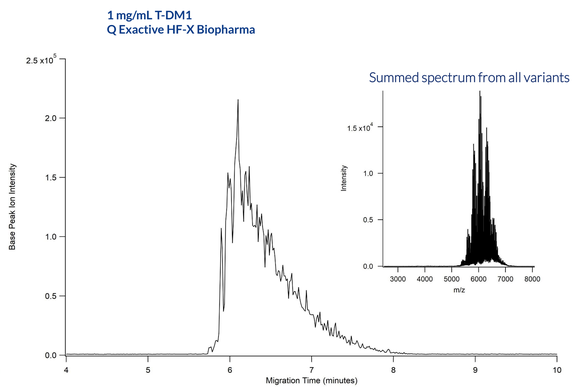


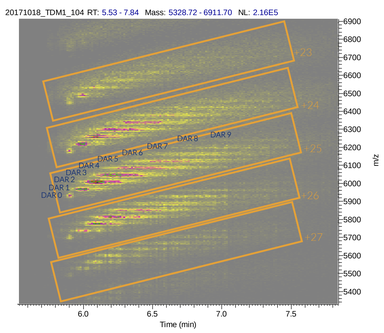


Microchip CE-MS separation of the NIST mAb under native conditions yields baseline resolution of the C-terminal lysine variants (2K, 1K and 0K), and resolution of one or more acidic variants. Resolution of these variants in the front end separation greatly improves the mass spec's ability to clearly identify the variants. The acidic variants have very small mass shifts, likely attributable to deamidations.

Native Analysis of Trastuzumab and T-DM1

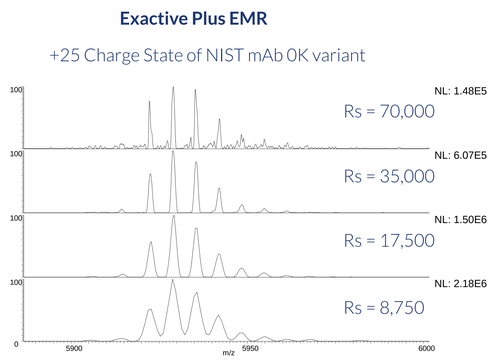


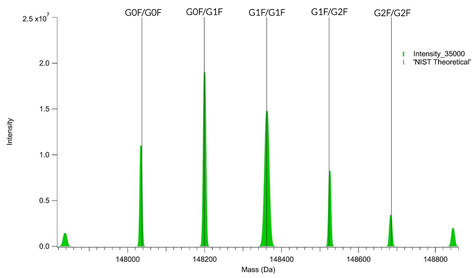




Conjugation of drug molecules onto the structure of trastuzumab creates a very complex group of variants. Without a separation, spectral overlap impedes the mass spec's ability to see all of the drug-antibody ratio (DAR) variant peaks. The 2D map view shows how the combined power of the separation and the mass spec can resolve all of the variants.

Mass Spec Performance





As others have previously shown, the orbitrap can achieve high resolution and mass accuracy of large intact proteins, but the signal intensity decays at higher resolution settings. We observed high mass accuracy at all resolution settings, with optimal glycoform resolution at a setting of 35,000. The plot above shows the deconvoluted spectrum acquired at 35,000 resolution compared to the theoretical masses. The measured mass error was 0.7 Da (~5 ppm)

Conclusions

The ZipChip platform has demonstrated the ability to resolve charge variants of native, intact mAbs and ADCs while also efficiently transferring them into the gas phase. Orbitrap mass specs with the capability of efficiently transporting large ions to the mass analyzer yield highly accurate and sensitive mass spec characterization of these ions. The peak profiles generated by the native ZipChip separation correlate very well with traditional charge variant separation techniques, such as isoelectric focusing, and ion exchange chromatography. The fast analysis times and easy set up of this system make this technology a simple and powerful addition to the biopharma toolkit.

References:
1. N.G. Batz et al., *Anal. Chem.* 2014, *86*, 3493-3500.
2. E.A. Redman et al., *Anal. Chem.* 2015, *87*, 2264-2272.
3. E.A. Redman et al., *Anal. Chem.* 2016, *88*, 2220-2226.