Charge Variant Mass spectrometry for Analysis of biotherapeutics with Orthogonal and Fractionated sampling

Xiaojing Shen, Ilker Sen, Krisztina Radi, St John Skilton, Baburaj Kunnumal, Kefei Wang

[1]Bio-Techne, 3001 Orchard Parkway, San Jose, CA 95134; [2]Protein Metrics, Boston, MA.

Summary

Aim: A direct comparison between LCMS and icIEF charge variant analysis with visual outputs to facilitate orthogonal analysis.

This poster shows a workflow designed to take as input the information from fractions from a Maurice system into the Byos® workflow that superimposes icIEF data and LC-MS data

Samples were of a Monoclonal-like Antibody (NISTmAb) with fractions isolated on an advanced icIEF system, followed by analysis by mass spectrometry

The combination of data streams provided interactive projects for LCMS users, and automated association of fractions from icIEF and peptide maps. The workflow highlighted discrepancies or similarities for each technique

Introduction

Imaged capillary isoelectric focusing (icIEF) is routinely performed for charge variant characterization of biotherapeutics but is incompatible with electrospray mass spectrometry. Advances in icIEF fractionation makes it possible to collect charge variant fractions offline for mass spectrometry characterization. How do users cope with the choice of a complex/ expensive linking of icIEF to Mass Spectrometry, or a tedious sample-by-sample offline analysis?

Here we demonstrate an intelligent and automated approach to directly correlate charge profile from icIEF to MS data from the analysis of the collected fractions. Despite different LC and iCIEF separation mechanisms, the charge profiles from the two techniques can be superimposed and related to each other mathematically by 'reconstructing' the variant profile and displaying it on top of the LCMS profile. Direct comparison is highly desirable in any protein characterization environment because MS provides much deeper understanding of composition, but icIEF alone is ideal for routine.

Methods

icIEF analysis and fractionation

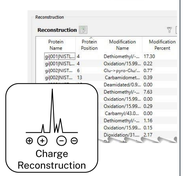
icIEF fractionation was carried out on a MauriceFlex system (Bio-Techne) on NISTmAb samples. The charge fractions were verified for identity and purity before being pooled for peptide mapping analysis on a High-Resolution Mass Spectrometer (Thermo Fisher).

Peptide mapping

Pooled fractions were digested with In-Solution Tryptic Digestion Kit (Thermo Fisher) according to its instruction. After the digestion was completed, the samples were lyophilized by SpeedVac. The digested samples were lyophilized and reconstituted in 40 µL 5 mM ammonium acetate solution.

Charge Reconstruction

The Byos charge reconstruction workflow automates processing of the acquired raw peptide mapping data and reconstructs in-silico a table from the peptide mapping results showing proportion modified against residue number. The icIEF fractions are plotted as traces over a pI scale with 'anchor' points at the apex of the peaks. A pKa value of 2.6 was used for sialic acid, and a translation of 1.35 pI units was applied to find the best match to the raw data and theoretical peaks.



Fractionation and Reconstruction

Figure 1 displays the reconstruction (blue) using the combined information from all fractions superimposed on the peak profile, correlated to the LCMS Peptide mapping results. Visually the results display a discrepancy between the relative proportions of species for almost all peaks. To investigate each one, the fractions are analyzed in turn. Overall, Oxidation, deamidation and glycosylation make up the bulk of the discrepancy, although the effect of Lysine on the Intact antibody would not be noticed at the peptide map level.

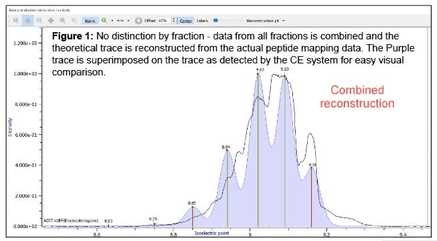


Figure 1: No distinction by fraction - data from all fractions is combined and the theoretical trace is reconstructed from the actual peptide mapping data. The Purple trace is superimposed on the trace as detected by the CE system for easy visual comparison.

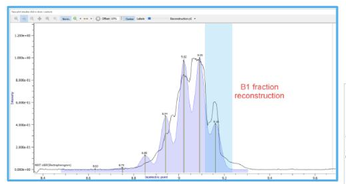
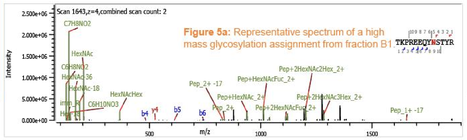


Figure 5: Basic Fraction B1 which correlates overall with an increase in basic species

Figures 5 and 6: cover the basic charge variant fractions B1 and B2. Mostly identified as 1 and 2 c-terminal Lys, respectively, as established by the intact mass analysis (data not shown here). The spectral evidence for glycosylation assignments is good in all fractions (Figure 5a).



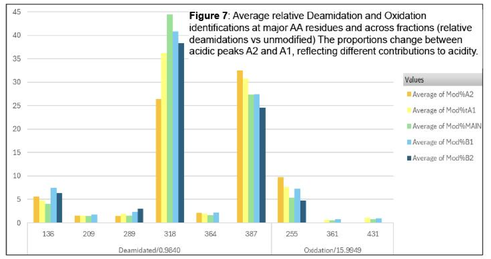


Figure 7: Average relative Deamidation and Oxidation identifications at major AA residues and across fractions (relative deamidations vs unmodified) The proportions change between acidic peaks A2 and A1, reflecting different contributions to acidity.

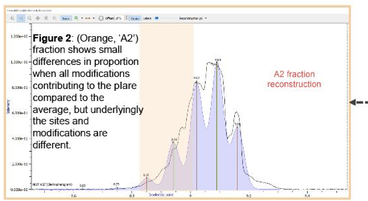


Figure 2: (Orange, 'A2') fraction shows small differences in proportion when all modifications contributing to the plare compared to the average, but underlyingly the sites and modifications are different.

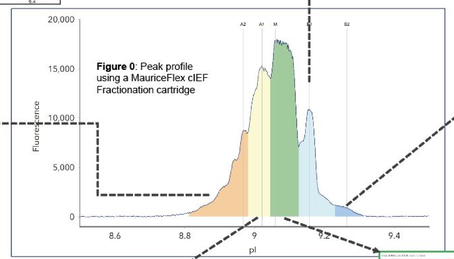


Figure 0: Peak profile using a MauriceFlex cIEF Fractionation cartridge

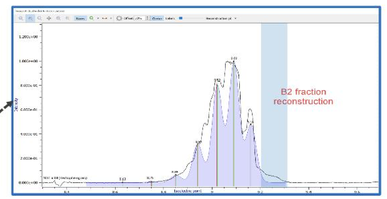


Figure 6: tailing final fraction B2 is much lower in abundance and some modifications identified in other fractions were not identified here (e.g.: Ammonia loss, low level deamidations, oxidations – see Fig.7 for selected examples)

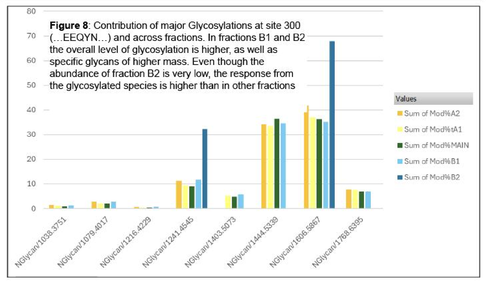


Figure 8: Contribution of major Glycosylations at site 300 (…EEQYN…) and across fractions. In fractions B1 and B2 the overall level of glycosylation is higher, as well as specific glycans of higher mass. Even though the abundance of fraction B2 is very low, the response from the glycosylated species is higher than in other fractions

In **Figure 2** the relative proportions at pI value 8.85 and 8.94 in fraction A2 are higher than the peak at pI value 9.02. Compared to other fractions, higher proportions of deamidations at sites 318, 364, 387, and lower proportions of Dethiomethyl at residues 32 and 431, and overall lower Ammonia loss were measured in the peptide map (Figure 7).

Figure 3 highlights fraction A1. The relative proportion of species at pI value 9.02 and 9.09 on the reconstruction trace changed for the A1 and Main peaks. This change probably correlates with the change of oxidation and deamidation proportions as illustrated by Figure 7 (Yellow and orange bars).

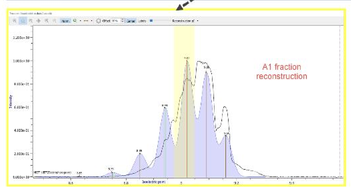


Figure 3: A1 is the most abundant acidic fraction and the peptide map coverage is higher for this fraction than for A2.

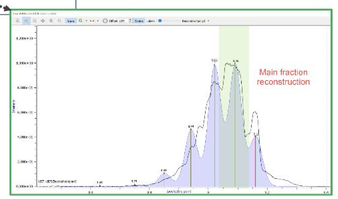


Figure 4: Main and acidic fractions show similarities reflected in the peptide mapping

Conclusions

Some modification proportions do not change significantly across the fractions, but glycosylations of certain types (higher mass, Fucosylated) increase dramatically in abundance in basic fractions.

Fraction A1 and A2 reconstruction can be correlated with modifications that provide acidity.

When looking at overall intensities of the peptide responses the higher concentration fractions have more identifications overall – unsurprisingly. This finding can help with method development for low abundance species.

The workflow presents a straightforward mechanism to directly compare orthogonal techniques.