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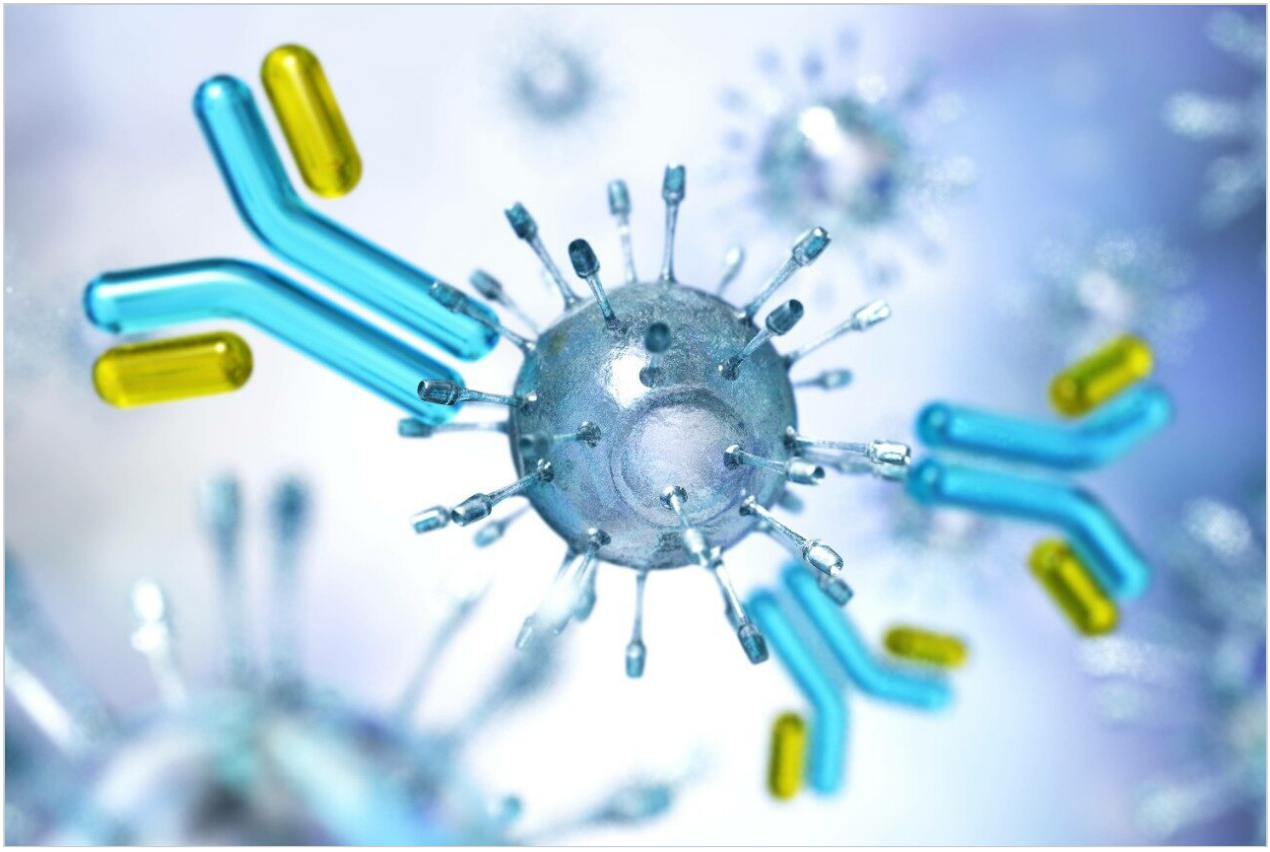
Applikationsbericht

mAb Subunit Analysis Using the SYNAPT XS High Resolution Mass Spectrometer

Henry Shion, Scott J. Berger, Ying Qing Yu

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

The SYNAPT XS is a versatile high resolution mass spectrometer that has the flexibility for routine attribute analysis as well as advanced characterization (e.g., HDX, CIU, and other higher order structure approaches) of biopharmaceuticals. Towards these aims, the SYNAPT XS provides a variety of acquisition and fragmentation modes that scientists can utilize to achieve their analytical objectives.

When analyzing monoclonal antibody (mAb) IdeS digested subunits, we compared the experimental results for data acquired under the four different mass resolution modes: sensitivity [Rs ~12,500], resolution [25,000], high resolution [56,000], and enhanced resolution [75,000]. In this study, it was found that all resolution modes generated high quality data, with similar low ppm mass accuracy and consistent relative product variant abundance, when processed using MaxEnt1 charge state deconvolution. In addition, we demonstrated that the BayesSpray[2] deconvolution algorithm can be used to obtain even higher mass accuracies from monoisotopic resolved mass spectra obtained from acquisition using the enhanced resolution mode.

Benefits

- SYNAPT XS – a versatile and flexible mass spectrometer for both routine analysis and advanced characterization for biopharmaceuticals
- All resolution modes provide similar low ppm mass accuracy and consistent relative abundance for identified components (e.g., glycoforms) using the MaxEnt1 deconvolution processing approach for mAb subunit analysis
- The enhanced resolution mode enables monoisotopic resolution of the 25 kD IdeS mAb subunits, and BayesSpray deconvolution processing yields even better mass accuracy, with a requirement for higher sample loadings

Introduction

The SYNAPT XS is a flexible tool for biotherapeutic characterization that provides a large variety of acquisition modes, fragmentation modes, and selectable resolution options that users can choose to optimize biopharmaceutical analytical applications. Routine analysis such as intact mass, native MS,[1] protein subunit,

peptide mapping, released glycan, and oligonucleotide analysis are capabilities of the SYNAPT XS platform. More advanced questions, those beyond primary structure, such as HCP (host cell protein), Top-Down protein fragmentation, HDX (Hydrogen-Deuterium Exchange), and CIU (Collision Induced Unfolding) mass spectrometry analysis are among the more advanced techniques that make use of the higher resolution and ion mobility capabilities of the SYNAPT XS.

In this study, we compared the experiment results with data acquired under four different resolution modes: sensitivity [Rs 12,500], resolution [25,000], high resolution [56,000], and enhanced resolution [75,000] for analyzing the ~25 kD monoclonal antibody (mAb) IdeS digested subunits. It was found that all the resolution modes generated accurate mass data with comparable mass accuracy (when processed using MaxEnt1 for deconvoluton) and relative abundance of the identified components. In addition, we demonstrated the BayesSpray deconvolution algorithm can be used to improve mass accuracy on monoisotopic resolved mass spectra obtained using the highest resolution mode (enhanced resolution).

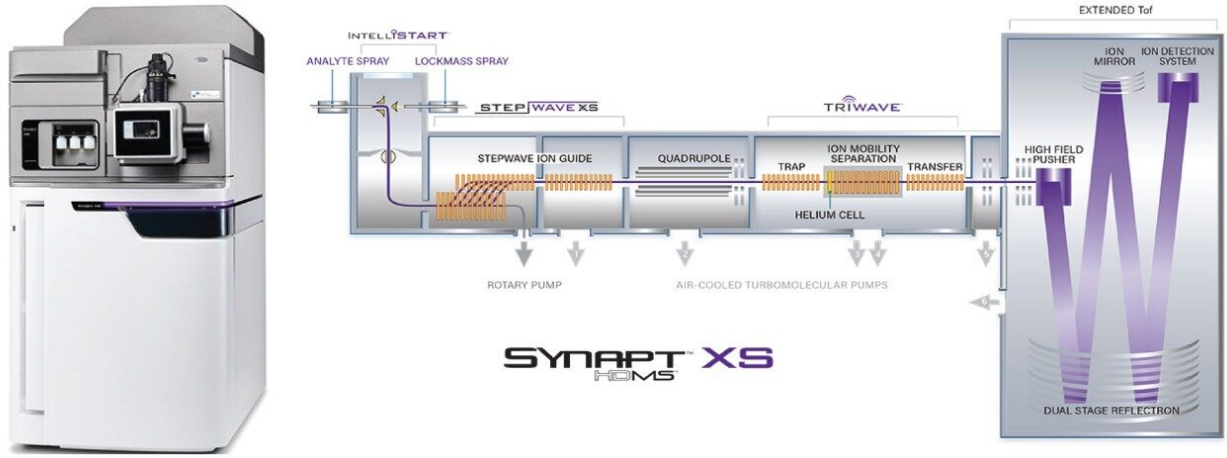


Figure 1. SYNAPT XS System (left) and schematic of the SYNAPT XS System (right).

Results and Discussion

Impact of Mass Resolutions on Charge Deconvolution Subunits

In this study, we report on RP LC-MS analysis of mononclonal antibody (mAb) IdeS digested subunits run on the SYNAPT XS System. Samples used were 1 NISTmAb subunits standard (Waters p/n 186008927 <

https://www.waters.com/nextgen/us/en/shop/standards--reagents/186008927-mab-subunit-standard.html >) and 2 Trastuzumab subunits that underwent forced degradation (oxidation) before IdeS digestion. We compared the sensitivity, relative variant abundance, and mass accuracy for the scFc (single chain Fc), LC (light chain), and Fd subunits from data generated using the four different resolution modes (sensitivity, resolution, high resolution, and enhanced resolution). In addition, we evaluated the potential benefits of using BayesSpray deconvolution algorithm for monoisotopic resolved data obtained in the enhanced resolution mode.

The inlet LC system used with the SYNAPT XS QTof was an ACQUITY UPLC I-Class PLUS. The mobile phases were: (A) 0.1% formic acid (FA) in DI water and (B) 0.1% FA in acetonitrile. The gradient of mobile phase B was from 25% to 35% over 10.5 mins and total runtime was 20 mins for each injection. The separation of subunits was carried out (80 °C) using the Waters BioResolve RP mAb Polyphenyl Column, 450 Å, 2.7 µm, 2.1 mm x 50 mm (p/n 186008944 < https://www.waters.com/nextgen/us/en/shop/columns/186008944-bioresolverp-mabpolyphenyl-column-450a-27--m-21--mm-x-50--mm-1-p.html>). The loading on the column for each injection was 0.4 µg of the sample. The SYNAPT XS was operated in the ESI positive mode using sensitivity, resolution, high resolution, or enhanced resolution modes. The capillary voltage was set at 2.0 kV, sampling cone at 50 V and the source office offset at 30 V for all analyses. The source temperature and desolvation temperature were maintained at 125 °C and 400 °C respectively. The desolvation gas flow was at 800 L/h, cone gas at 50 L/h, and nebulizer gas at 6.5 Bar. The system was controlled by MassLynx (version 4.1.2). All data were imported to and then processed within waters_connect (1.9.7) using MaxEnt1 and BayesSpray deconvolution processing within the UNIFI Intact Mass data processing workflow.

Figure 2 shows the TIC (total ion current) chromatograms from the data acquired in the sensitivity, resolution, high resolution, and enhanced resolution modes for the NISTmAb IdeS digestion subunit standard (Waters p/n 186008927 <https://www.waters.com/nextgen/us/en/shop/standards--reagents/186008927-mab-subunit-standard.html>). The three major ~25kD subunit fragments of scFc, Light Chain (LC) and Fd from the sample are well separated and with consistent retention times. TIC response varies for the four mass resolution modes, as ion transmission decreases when moving from lower to higher resolution modes. This is a typical observation as the optics required to generate a narrower ion beam and longer TOF detection pathlengths reduce transmission efficiency. Response in the sensitivity mode is about 2x of the resolution mode; 20x of the high resolution mode; up to roughly 80x greater than the enhanced resolution mode.

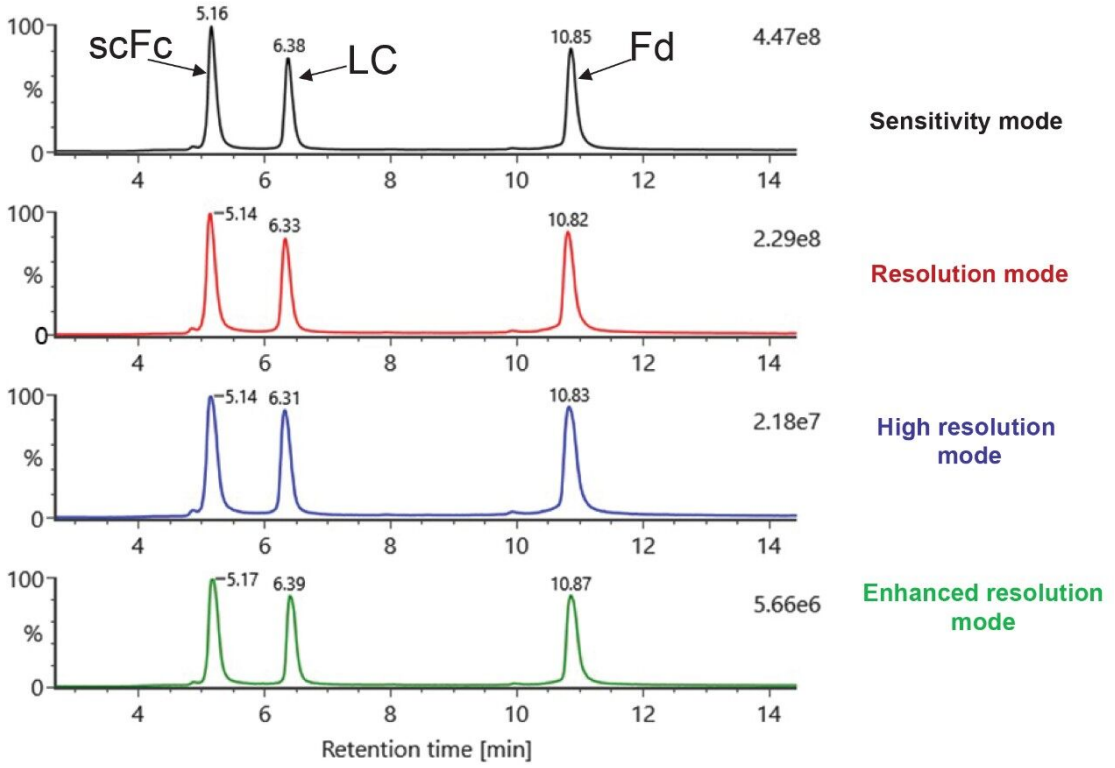


Figure 2. TIC comparison for data acquired amongst sensitivity, resolution, high resolution, and enhanced resolution modes (with mass resolution of 12,500, 25,000, 56,000, and 75,000 respectively) demonstrating the inverse relationship between MS sensitivity and Tof detection resolution.

The combined raw and MaxEnt1 deconvoluted spectra show the same trend (data not shown). While MS response is observed to vary with resolution, there is no effective mass accuracy difference for the identified components amongst the four resolution modes (data not shown) following MaxEnt1 deconvolution. Assigned components for all four modes are all less than 15 ppm from predicted average masses, with most well within the <10 ppm range.

Figure 3 contains relative percentage of major proteoforms from NISTmAb subunits obtained from applying the four MS resolution modes. As demonstrated in the mass spectral plot, the relative percentage for the main proteoforms is consistent, independent from acquired MS resolution. The combination of low ppm mass accuracy and consistent results across all modes suggests that for subunit analysis, sensitivity mode should be recommended as the default choice for resolution mode. It will provide the users with the most sensitivity, excellent mass accuracy, and consistent relative abundance for Ides subunit variants (identified to about as low as 3% in relative abundance level in this sample).

NISTmab subunit major identified component relative abundance table					
NISTmAb subunit major Identified componets	Expected average mass (Da)	Protein Mod-MS (%)			
		Sensitivity mode	Resolution mode	High resolution mode	Enhanced resolution mode
LC	23127.51	95.5	95.5	95.6	95.8
LC+Gly	23289.65	4.5	4.5	4.4	4.2
FC+Gly+G0F N	25235.98	40.9	40.6	39.8	41.1
Fc+Gl+ G0F-GlcNAc N	25032.78	3.1	2.9	3.2	3.0
Fc+Gly+G1F N	25398.12	39.7	40.0	39.9	39.3
Fc+Gly+G1F-GlcNAc N	25194.92	3.7	3.5	3.8	4.7
Fc+Gly+G2F N	25560.26	10.1	10.3	10.5	10.1
Fc+Gly+G2F N+Alpha-Gal	25722.40	2.6	2.6	2.8	1.9
Fd+Pyrog Q N-Term	25688.86	96.5	96.5	96.4	96.2
Fd+Pyrog Q N-Term+Gly	25851.00	3.5	3.5	3.6	3.8

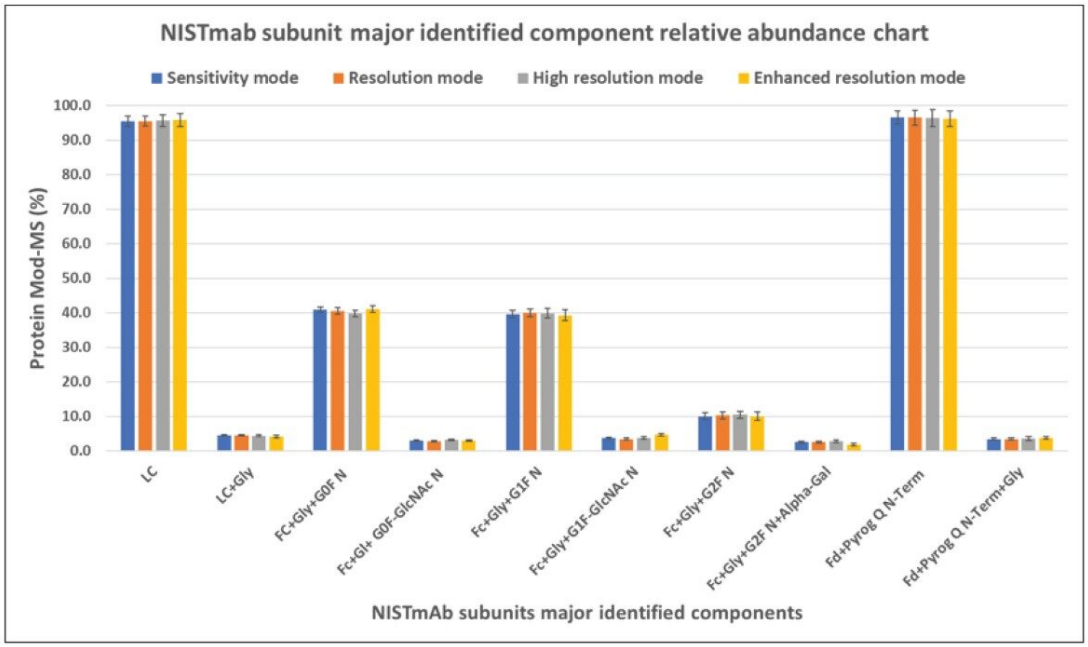


Figure 3. Identified NISTmAb Fc subunit glycoforms plus Fd and light chain variants demonstrate consistent relative response across repeated injections and all MS resolution modes.

BayesSpray Charge Deconvolution for Enhanced Mass Resolution

The enhanced resolution mode has a specification of 75,000 FWHM measured on the $(M+6H)^{6+}$ isotope cluster of bovine insulin (at *m/z* 956) can generate isotopic resolution on the 25kD mAb IdeS digested subunits. To further investigate the enhanced resolution mode results, we ran an Ides digested Trastuzumab subunit sample at a loading of 4.0 µg on column. The Trastuzumab was incubated at 37 °C for four hours

with 0.03% of hydrogen peroxide (H_2O_2) prior to digestion. Comparing deconvoluted spectra derived from MaxEnt1 and BayesSpray deconvolution processing, it was found that the BayesSpray deconvoluted spectra gives lower and tighter mass errors than those obtained from MaxEnt1 processing (Figure 4.) For this analysis, MaxEnt1 peak width was chosen to generate an average mass spectrum, while processing using the BayesSpray algorithm was optimized for returning the monoisotopic mass. While the results indicate superior mass accuracy from the BayesSpray deconvolution approach, these differences would not yield practical differences for interpretation of typical subunit spectra.

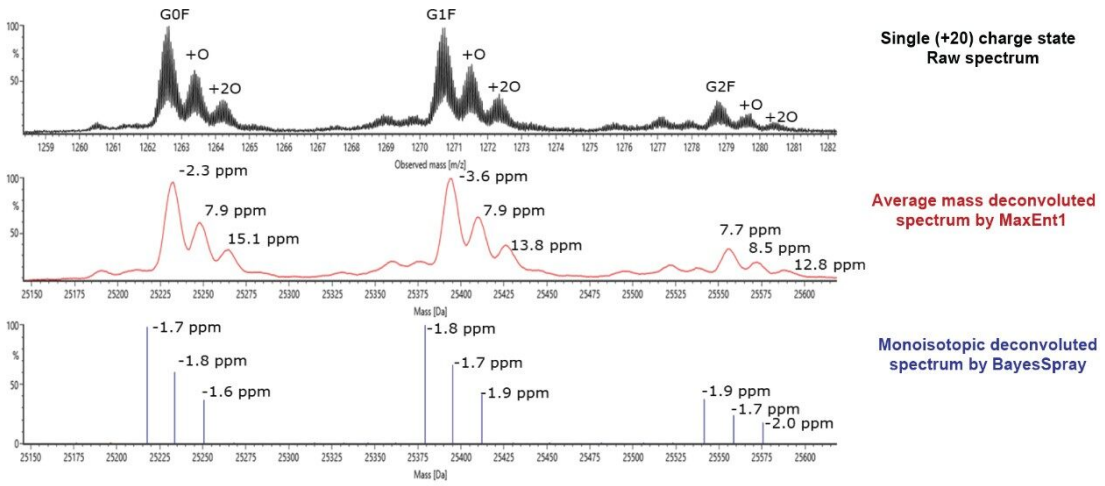


Figure 4. Spectra comparison for the oxidized Trastuzumab scFc subunit fragment before and after charge state deconvolution. Top: single (20+) charge state raw spectrum of monoisotopic resolved scFc subunit peaks showing unmodified, singly, and doubly oxidized variants of the three major glycoforms. Middle: average mass deconvoluted MaxEnt1 deconvoluted spectrum with superimposed centroided average mass error. Bottom: monoisotopic centroided spectra obtained from BayesSpray deconvolution superimposed with mass errors relative to the calculated monoisotopic mass.

Conclusion

The SYNAPT XS is a versatile mass spectrometry system that offers the flexibility for routine and advanced biotherapeutic analysis using multiple mass resolution modes. When analyzing monoclonal antibody (mAb) IdeS digested subunits, sensitivity data acquisition mode is recommended to obtain the best MS sensitivity,

low ppm mass accuracy, while obtaining consistent relative abundance for digested components. While not required for a typical subunit-based analysis workflow, enhanced resolution mode generates monoisotopic resolved IdeS subunit spectra that have the potential to produce better mass accuracy (at <2ppm) when using BayesSpray charge deconvolution processing. The sample injection amount needs to be increased to offset the loss of sensitivity when using this mode.

References

1. Shion H, Quinn C, Berger S, and Yu Y. W. Analysis of Cysteine-Conjugated Antibody Drug Conjugates (ADCs) Using a Native SEC LC-MS Workflow on the SYNAPT XS. Waters Application Note 720007026EN <https://www.waters.com/nextgen/us/en/library/application-notes/2020/analysis-of-cysteine-conjugated-antibody-drug-conjugates-adcs-using-a-native-sec-lc-ms-workflow-on-the-synapt-xs.html> . 2020 October.
2. Skilling, J and Richardson K. Method and System of Identifying a Sample by Analyising a Mass Spectrum by the Use of a Bayesian Inference Technique. EU patent EP2558979A1, 2011.

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