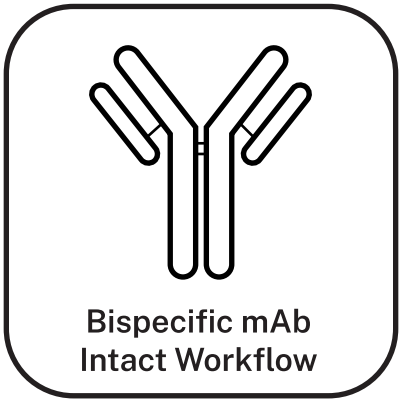
Highly Automated Analysis of Chain Shuffling in Bispecific Antibodies

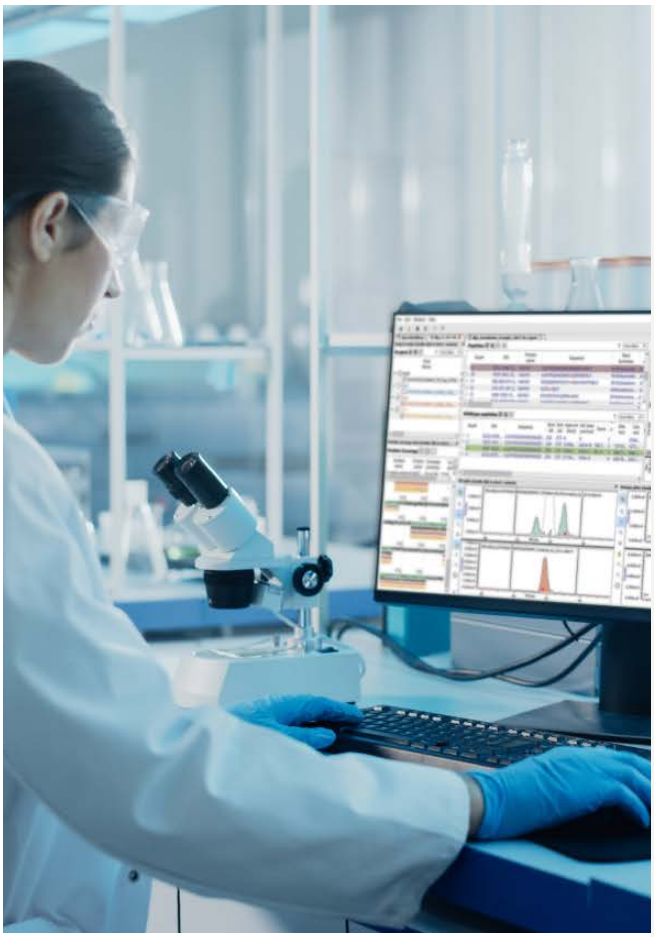
PROTEIN METRICS by Dotmatics

Dotmatics

Summary

- This Application Note describes an intact mass approach to investigate chain shuffling in bispecific antibodies.
- The Intact workflow in Byos® provides a streamlined method for automatic identification and assessment of bispecific antibody structure.
- The parsimonious charge deconvolution algorithm is well suited to resolving highly heterogeneous mixtures that result from the undesired matching of the antibody chains.
- Enhanced visualization tools facilitate manual inspection and validation of complex intact data sets.





Introduction

Monoclonal antibodies usually contain two identical heavy chains and two identical light chains, but Hetero-IgGs and other bispecific antibodies contain different heavy and/or light chains[1]. Methods for expressing hetero-IgGs in a single cell-line can produce undesired “shuffled” combinations. For example, an undesired product can contain different heavy chains but identical light chains. To enable molecule design optimization, as well as expression/purification optimization of these bispecific constructs, mass spectrometry is needed to accurately and efficiently identify and quantify desired and undesired molecules. Here we describe an automated system that deconvolves intact-mass spectra, labels and quantifies desired and undesired peaks and prepares reports for expert human inspection. The system relies on a “parsimonious” charge deconvolution algorithm that produces fewer artifacts and preserves peak shapes compared to other algorithms.

Materials and methods

Mass spectrometry

Non-reduced LC-MS data of hetero-IgG molecules was acquired on an ESI-TOF system. Method details were published previously[2]. Prior to LC-MS analysis N-glycans were removed by PNGase F digestion: In short ~ 20µg of proteins were diluted with PBS and 0.6 µL of PNGase F (New England Biolab), incubated overnight at 37 °C. Approximately 5µg of protein was analyzed using an Agilent Technologies 1100 capillary HPLC connected to an Agilent Technologies 6224 ESI-TOF mass spectrometer.

Data processing

The parsimonious charge deconvolution algorithm, used for this dataset, can deconvolve wide m/z and m ranges, while producing minimal artifacts. It requires unambiguous evidence for each of the masses in the deconvolved mass spectrum, meaning for example for every peak present in the deconvoluted spectrum there will be a clearly observed and well separated m/z peak series in the raw data MS spectrum. The algorithm is implemented in the Intact Mass workflow and can process any MS vendor raw file format while taking advantage of several useful features, including batch processing and a multi-sample viewer enabling efficient review and visualization of the analyzed samples for fast screening in an automated manner. Data analysis was performed within Byos version 4.2.

Results and Discussion

A hetero-IgG is a format of bispecific antibody. Due to its complexity, expressing it in a single cell-line can theoretically produce many impurities (Figure 1). Optimization of the production process can greatly improve the impurity profile. Mass spectrometry plays a critical role in this optimization process[3].

Figure 1: Hetero-IgG and some of its possible impurities.

The simple concept of assigning certain mass as “undesired” enables the software to match not only the correctly formed hetero-IgG molecule, but also the various mis-formed molecules (Figure 1). Together with an additional category of “unexpected” mass, the software can use business rules to assign each sample to three auto validation categories (Pass, Fail, and Review), color coded in an intuitive “traffic light system” (Figure 2)

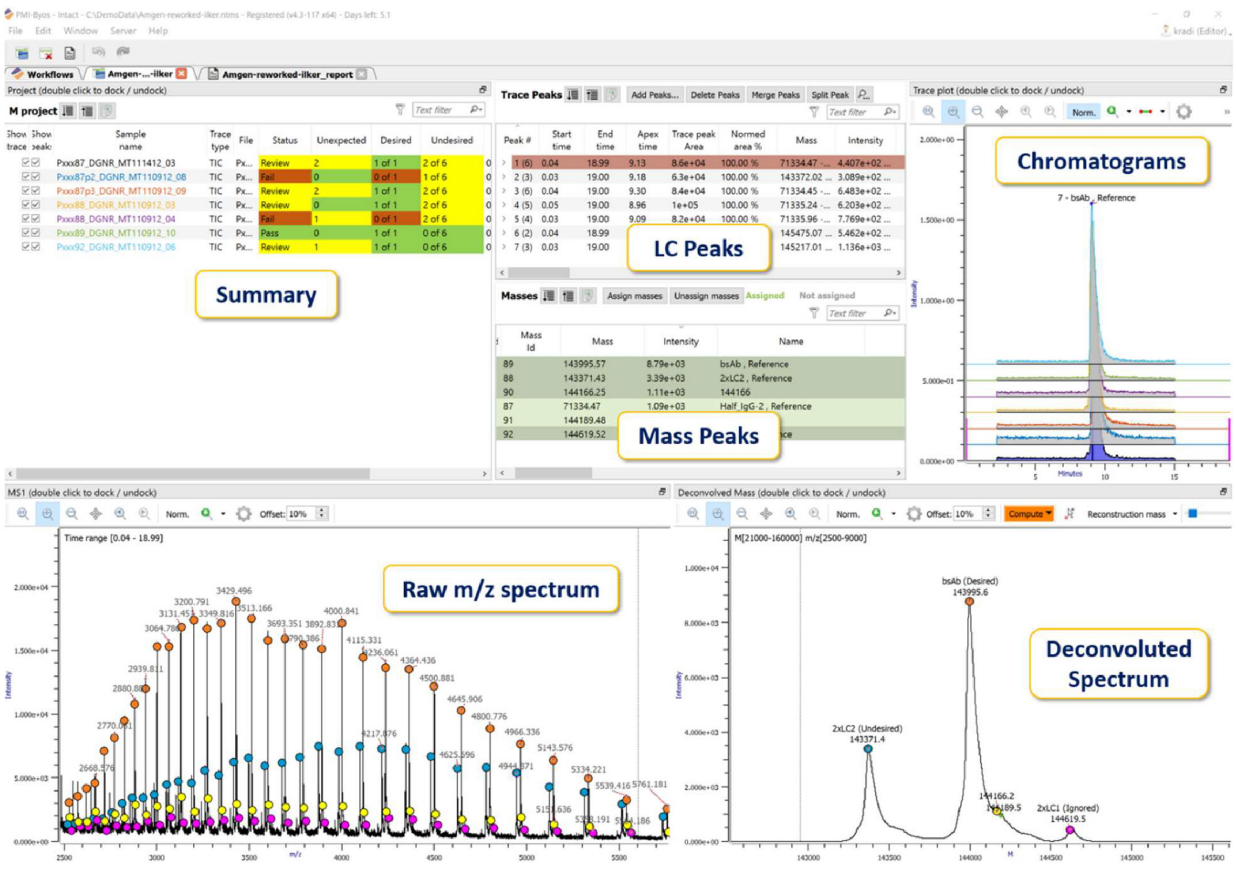


Figure 2: Multi-Sample Viewer

Multi-sample user interface. “Deconvolution dots” are used to mark the m/z signals that contribute to the mass signal. Deconvoluted peaks are labelled as desired, undesired and ignored. The overall validation status for each sample is color-coded in Summary Panel.

Figure 3 shows the results of different CEX fractions that received validation status as “Pass” “Fail” and “Review”. The main species detected consists of one copy of each heavy chain (HC1 and HC2) and two copies of light chain two (2 x LC2). Mass spec data clearly showed that this fraction is not usable, and the status was automatically assigned as “fail”. While the “Review” score sample contains the expected form, but also shows the presence of some incorrect half IgG molecules. The multi-sample interface (Figure 2) enables inspection of the automatically detected and matched signals. After inspection, the analyst can change the assignment of individual ions as well as the overall “verdict” of the sample. The analyst can also capture the zoomed-in views of ions of interest and include them in PDF reports.

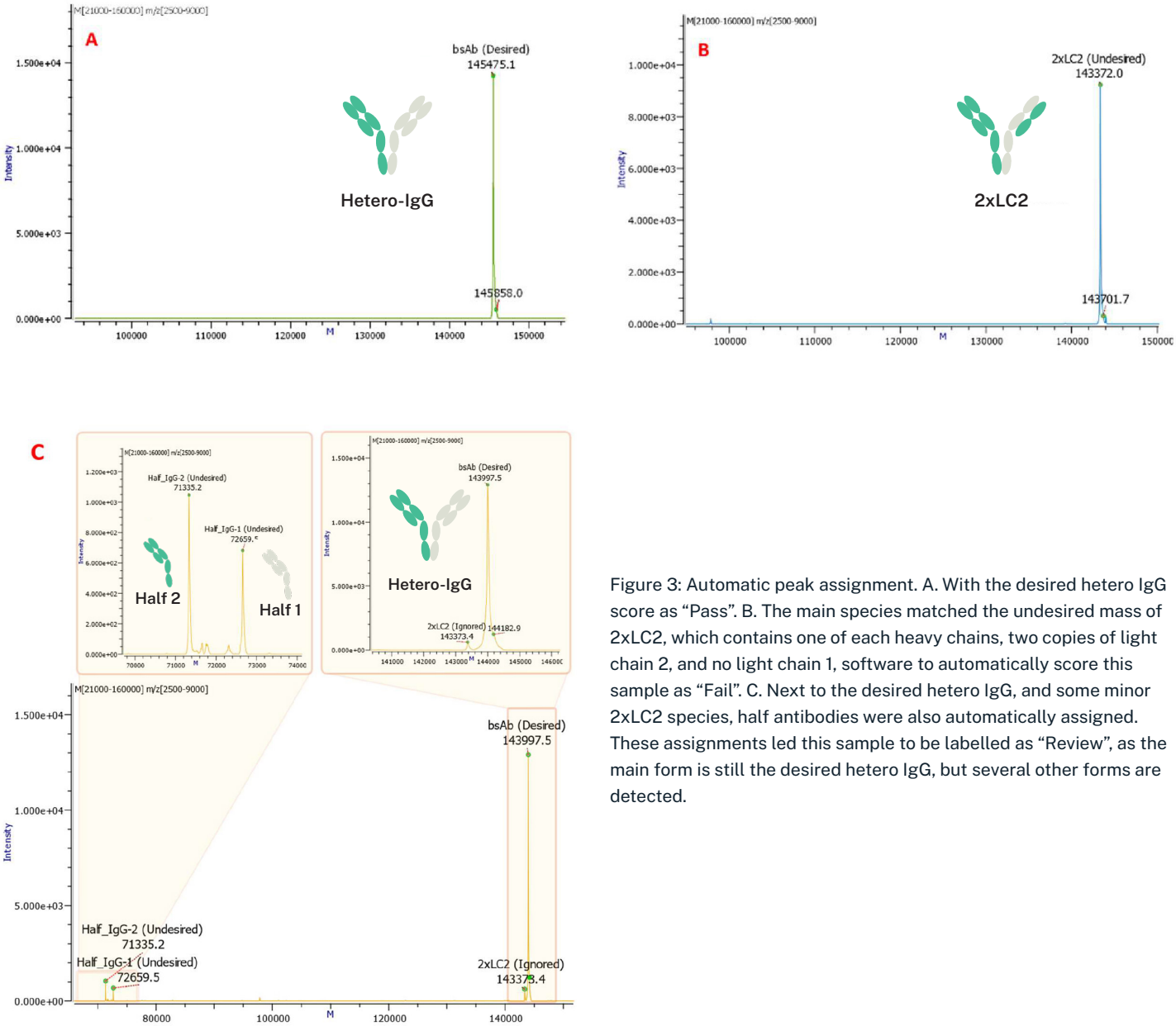
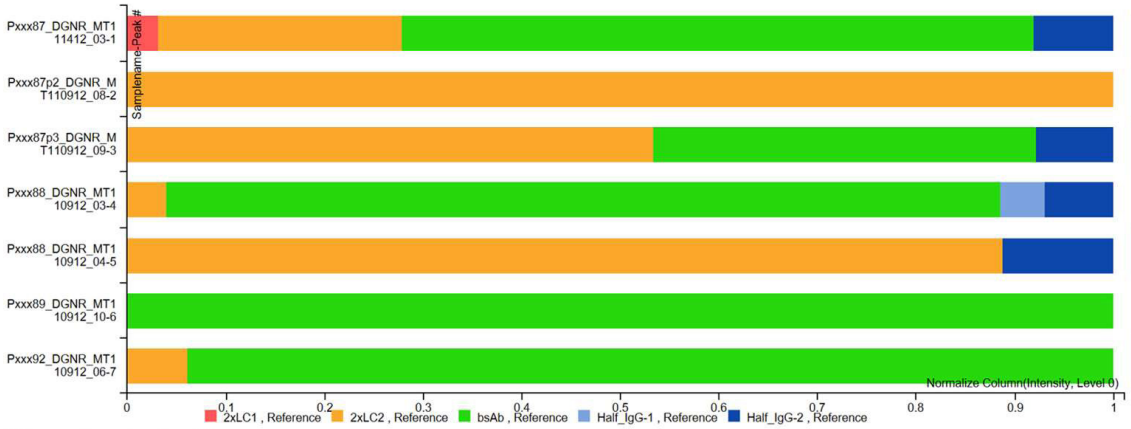


Figure 3: Automatic peak assignment. A. With the desired hetero IgG score as “Pass”. B. The main species matched the undesired mass of 2xLC2, which contains one of each heavy chains, two copies of light chain 2, and no light chain 1, software to automatically score this sample as “Fail”. C. Next to the desired hetero IgG, and some minor 2xLC2 species, half antibodies were also automatically assigned. These assignments led this sample to be labelled as “Review”, as the main form is still the desired hetero IgG, but several other forms are detected.

When reviewing the processed data and failed samples in more detail, the scientist can investigate the assigned peaks in the deconvoluted mass spectrum tab as shown in Figure 3. As all samples are processed in one batch, one can review all pass and fail categorized samples for more details, this allows a very easy and fast visualization of the assessment of conditions which could be successful during cell line development and optimization. The candidates which contain the expected form but also some undesired species are categorized as “Review” and the scientist can review the results and the annotated deconvoluted spectra to see more details of the mismatched or undesired species detected.

The automatic labelling for desired, undesired and ignored are setup with the customizable annotations tools through the spectrum tab settings icon, and it can be a great help for a quick visual assessment when the scientists need to manually review the samples categorized as Review due to the detection of mismatched structural components.

Sample name ↑	Name →	Half_IgG-2 , Reference (%)	Half_IgG-1 , Reference (%)	bsAb , Reference (%)	2xLC2 , Reference (%)	2xLC1 , Reference (%)	
Pxxx87_DGNR_MT111412_03		7.98		64.11	24.70	3.21	Review
Pxxx87p2_DGNR_MT110912_08					100.00		Fail
Pxxx87p3_DGNR_MT110912_09		7.73		38.86	53.41		Review
Pxxx88_DGNR_MT110912_03		6.86	4.48	84.59	4.07		Review
Pxxx88_DGNR_MT110912_04		11.10			88.90		Fail
Pxxx89_DGNR_MT110912_10				100.00			Pass
Pxxx92_DGNR_MT110912_06				93.81	6.19		Review



Normalize Column - Intensity, Level 0
Normalize type - Sum
Data axis: Row
Filter applied on: Protein name.

Figure 4: Report outputs for batch processed samples A. Typical pivot summary table showing relative abundances of the species detected. This comes with the standard caveat that lower mass species have much higher mass signal, and therefore half-antibody levels are over-estimated. B. Stacked bar chart showing relative amounts of species present, instantly visualizing if not the expected green species is the prevalent form in the analyzed sample. (The traffic light codes are added for visualization, but not shown like this in the generated report within Byos.)

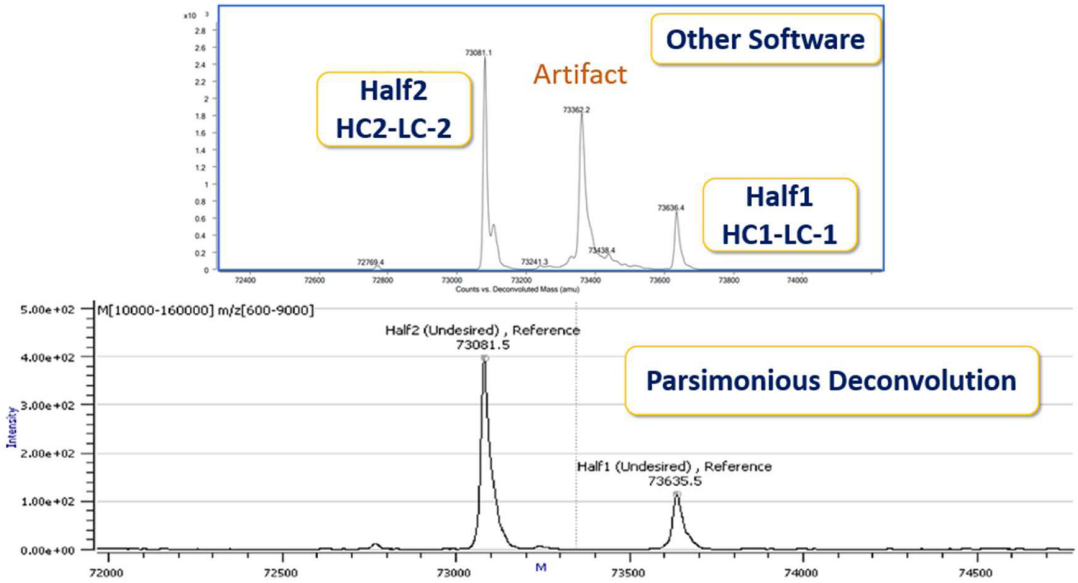


Figure 5: Real and Artifactual Half Antibody

One of the benefits of the parsimonious charge deconvolution algorithm is the lack of artifacts. Half-mass "harmonics" are frequently encountered and can introduce confusion in data analysis. The hetero-IgG molecule offers a model to study this artifact. Figure 5 showed one example where the parsimonious deconvolution software was able to suppress the artifactual half-antibody signal, while preserving signal from real half antibodies.

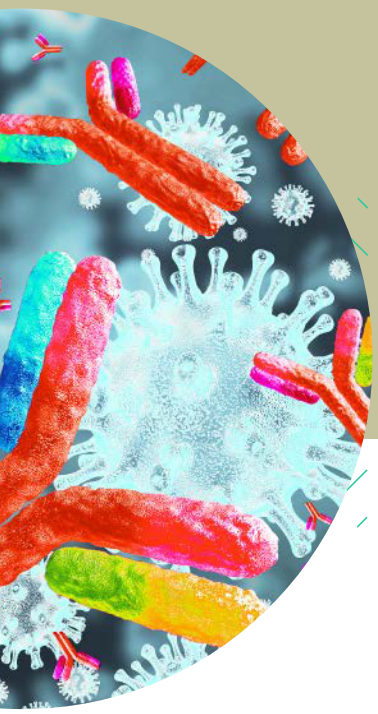
Conclusions

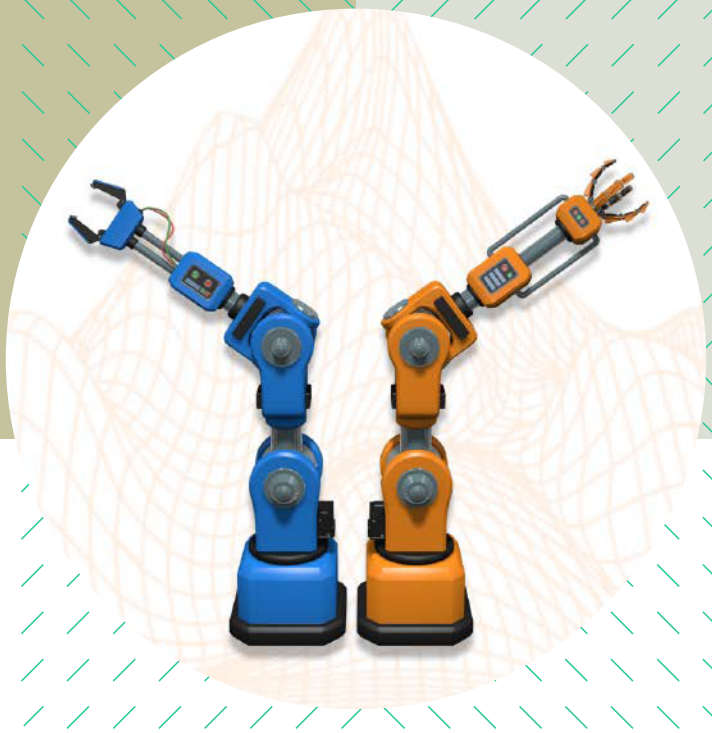
- Batch processing can automatically process large numbers of LC-MS runs, assign detected mass according to the provided mass table of expected and undesired masses and give an overall assessment of each sample based on the masses detected with a very helpful visual aid instantly showing samples as pass or fail
- The multi-sample view user interface enables review of the automatic assignments, and allows revision based on manual input.
- The parsimonious deconvolution produces less artifacts, notably half-mass artifacts. This feature is especially useful in hetero-IgG mass spec studies.
- This quick intact level MS analysis of bispecific antibodies shows how easy it is to achieve significant productivity gain in analyzing complex protein samples by mass spectrometry.

References:

1. *Brinkmann, U.; Kontermann, R.E.: The making of bispecific antibodies. MAbs. 9, 182-212 (2017)*
2. *Spahr, C.; Shi, S.D.-H.; Lu, H.S.: O-glycosylation of glycineserine linkers in recombinant Fc-fusion proteins: attachment of glycosaminoglycans and other intermediates with phosphorylation at the xylose sugar subunit. MAbs. 6, 904-914 (2014)*
3. *Yin, Y.; Han, G.; Zhou, J.; Dillon, M.; McCarty, L.; Gavino, L.; Ellerman, D.; Spiess, C.; Sandoval, W.; Carter, P.J.: Precise quantification of mixtures of bispecific IgG produced in single host cells by liquid chromatography-Orbitrap high-resolution mass spectrometry. MAbs. 8, 1467-1476 (2016)*

The authors would like to thank John Robinson, Iain Campuzano, Ling Liu, and Guna Kannan for helpful discussions.





Protein Metrics LLC.
Cupertino, California
USA
www.proteinmetrics.com
info@proteinmetrics.com

Dotmatics