

# High Throughput Data Processing and Comparison of Intact Mass Analysis using BPV Flex software 1.0.1

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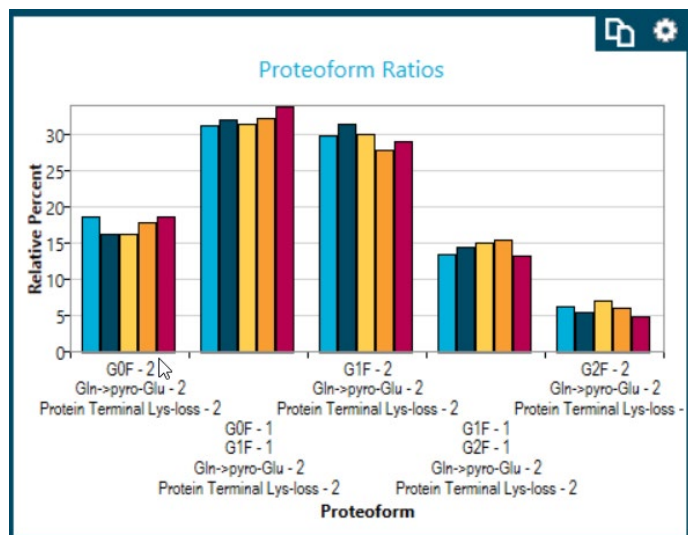
The analysis of monoclonal antibodies requires a wide range of approaches to characterize and track product attributes and correlate differences to activity assays. Of the commonly used assays, intact mass analysis is among the most common. The popularity of intact analysis stems from its ability to provide the molecular weight, high-level glycoform distribution, and other heterogeneity which are easily detected at the intact level.

Intact mass studies are frequently used throughout protein therapeutic development and as a platform assay. As this assay is used across products and projects throughout development, it is often important to review the results of current studies with previous results. Ideally, this is accomplished without the need for re-acquiring or reprocessing data. In addition, intact mass analysis is frequently used for high throughput studies due rapid analysis time, and limited sample preparation required.

## SCIEX X500B QTOF system



In this technical note we highlight the use of BPV Flex software 1.0.1 for high throughput analysis of intact mass data. Presented are how this solution enables an automated solution for high throughput data processing, and subsequent comparison, of results to track targeted quality attributes at the intact protein level. In addition, BPV Flex software 1.0.1 allows comparison of results from different studies to compare similarity and differences between batches of product over time. The powerful and flexible filtering capability enables users to focus on aspects of their study which are important for their work.



**Figure 1.** Comparison of glycoforms across multiple analysis of mAb samples.

## Key Feature of BPV Flex Software 1.0.1

- Refine and optimize processing parameters using an interactive interface
- Customize and save molecule information, processing parameters, and results independently to enable flexible processing and review of studies
- Powerful filtering of results using intuitive drag and drop sorting to focus on results that matter
- Undock and change result window layout based on user preference and save preferences for future use
- View and compare across analyses to guide method development and optimize processing parameters
- Directly compare raw and reconstructed data to rapidly investigate similarities and differences in samples

## Methods

**Sample Preparation:** NISTmAb was diluted to a concentration of 0.1 ug/ul using deionized water.

**Chromatography:** Separation was accomplished using an ExionLC™ system fitted with an Agilent PLRP-S column (2.1mm X 50mm, 300Å, 5µm) at 80°C using the gradient shown in Table 1. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in acetonitrile.

**Mass Spectrometry:** A SCIEX TripleTOF® 6600+ system with an TurboV® Ion source was used for data acquisition. Data was acquired using TOF-MS mode with intact protein mode (IPM) active. MS instrument conditions are listed in Table 2.

**Data Processing:** Data were processed using BPV Flex software 1.0.1 using the settings defined in Table 2.

**Table 1. LC Conditions**

Time (min)	%A	%B	Flow Rate ml/min
Initial	75	25	0.25
3.0	75	25	0.25
9.0	10	90	0.25
11.4	10	90	0.25
11.5	75	25	0.25
17.0	75	25	0.25

**Table 2. Reconstruction Parameters**

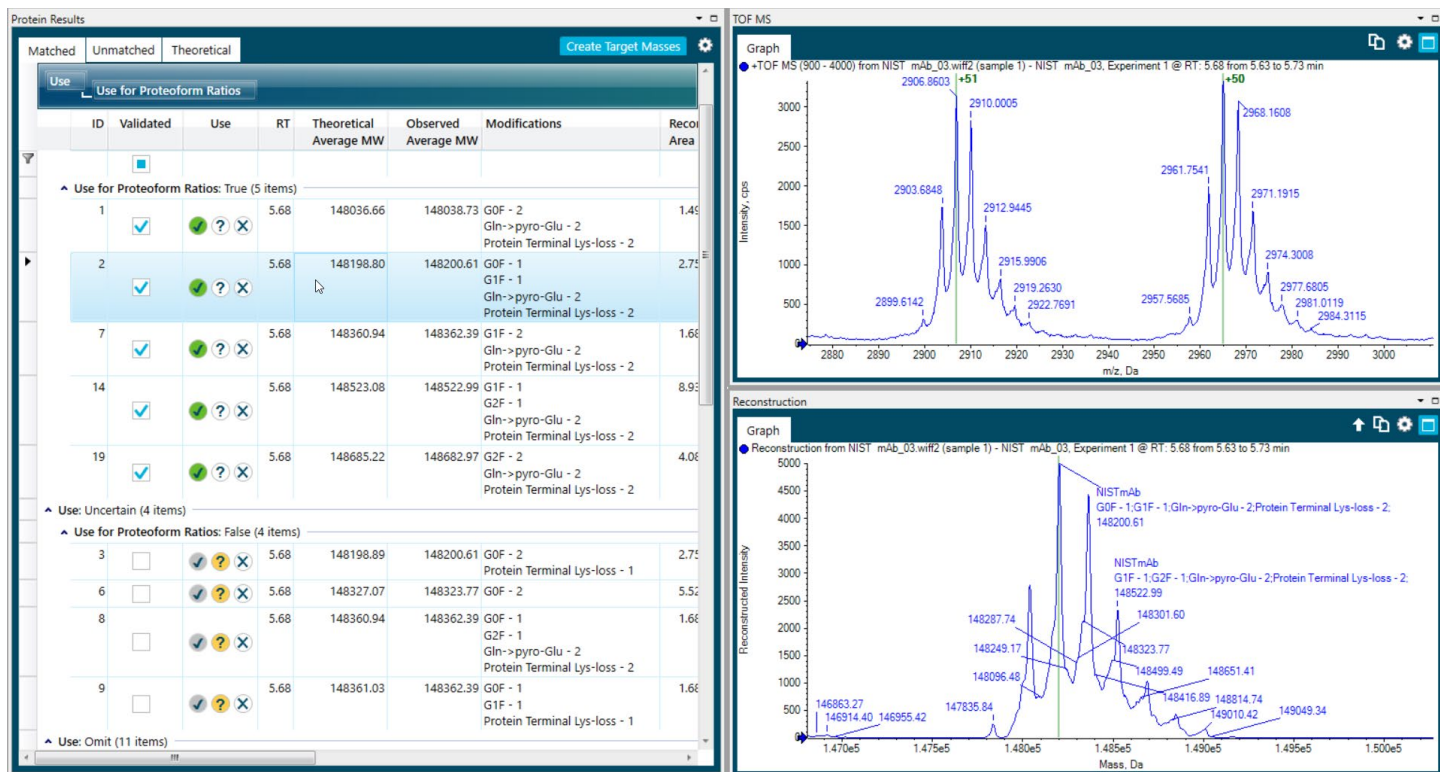
Parameter	Setting
Mass Range Selection	Mass Range automatically set by defined molecule
Peak Threshold	5%
Step Mass	1.0 Da
Iterations	20
Signal to Noise Threshold	≥20
Gaussian Smoothing	0
Resolution	9000

**Table 3. MS Parameters**

Parameter	Setting
Scan Mode	Positive
GS1	60
GS2	60
Curtain Gas	30
Temperature	500°C
Ion Spray Voltage	5500 V
Time Bins to Sum	80
Accumulation Time (ms)	0.5 sec
TOF Start Mas (Da)	400
TOF Stop Mas (Da)	4000
Declustering Potential	250.0
Collision Energy	10

## Results and Discussion

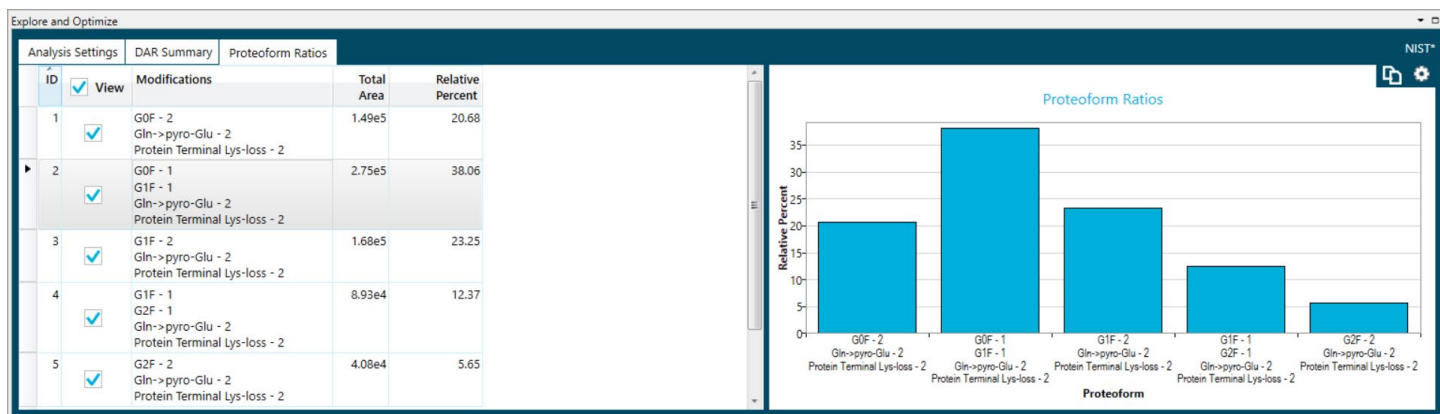
Processing of intact mass data begins with definition of the target molecule which includes the primary sequence, disulfide bond arrangement, and expected modifications and their location if known. Modifications can be positioned if desired, particularly if the defined molecule will be used in additional studies. Once the molecule is defined, the processing parameters are optimized to ensure an accurate result which is reflective of the input data. Parameters such as reconstruction resolution, matching tolerance, and input spectrum mass range are easily adjusted within the explore and optimize window in BPV Flex software 1.0.1. Additional parameters, if needed, are accessed through the advanced settings within the analysis parameters. Once the settings are defined, they may be saved as a parameter set which is easily applied to future studies to limit the required effort in assay definition. In addition, if multiple sets of conditions are desired, each parameter set is easily saved and may be applied to the same data file for comparison of their impact on results.



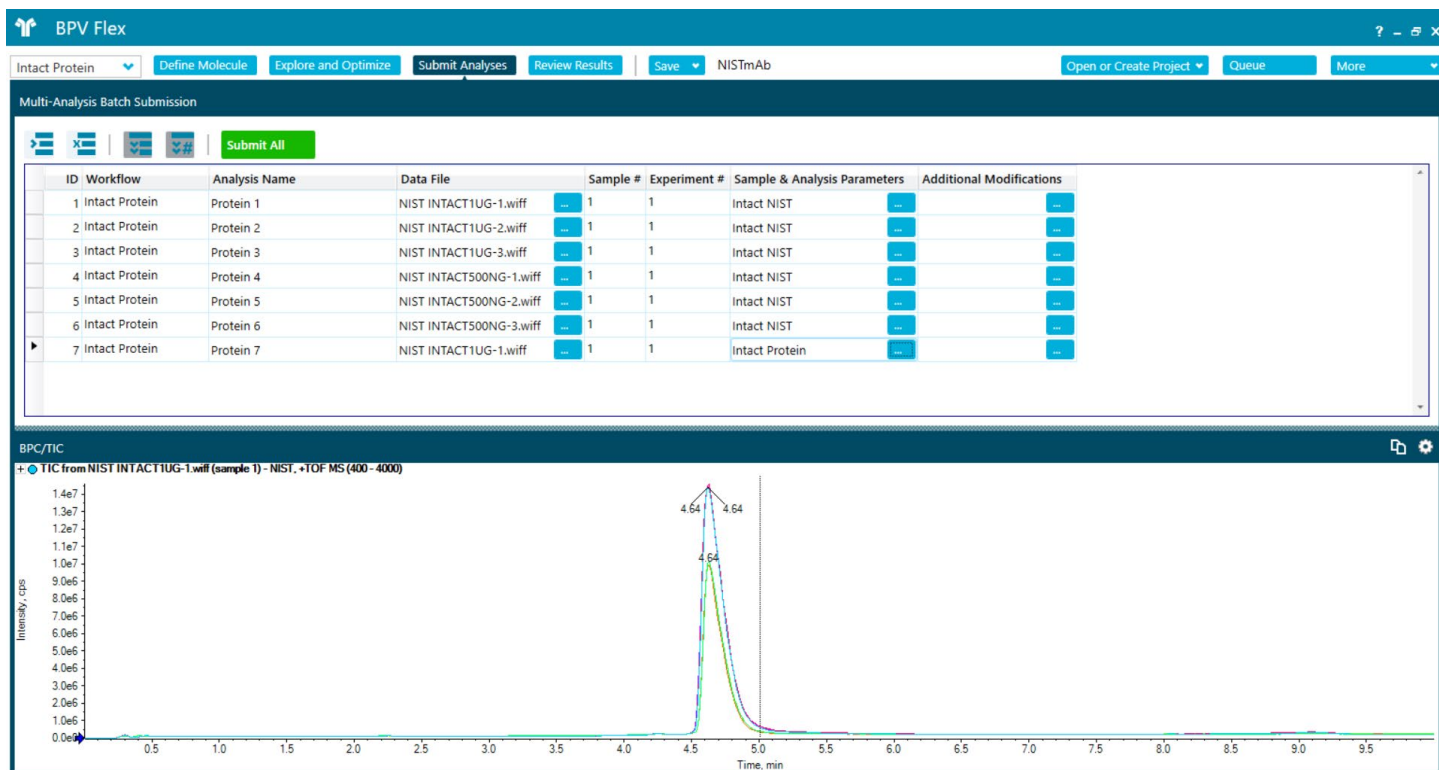
**Figure 2.** Review of processed data using flexible window arrangement in BPV Flex software 1.0.1. Each window may be rescaled, and the data are linked to expedite review.

The processed results are reviewed to assign the matches of interest for the study. For each matched component, the underlying raw data and reconstructed data correlate well. To expedite review, the protein results, raw data, and reconstructed data may be undocked from the review window to allow for evaluation of constituents and their corresponding underlying data, Figure 2. Undocking is particularly useful when using multiple monitors. Each matched component was flagged for

review, and the data was sorted using the interactive filtering criteria. This allows users to focus on components of interest for the calculation of proteoform ratios. During curation of results, proteoform ratios are plotted within BPV Flex software 1.0.1 to rapidly review the experimental results. Data is tabulated and may be grouped by modification or sample as shown in Figure 3. Once processing parameters are set, the project and preference may be saved for future use.



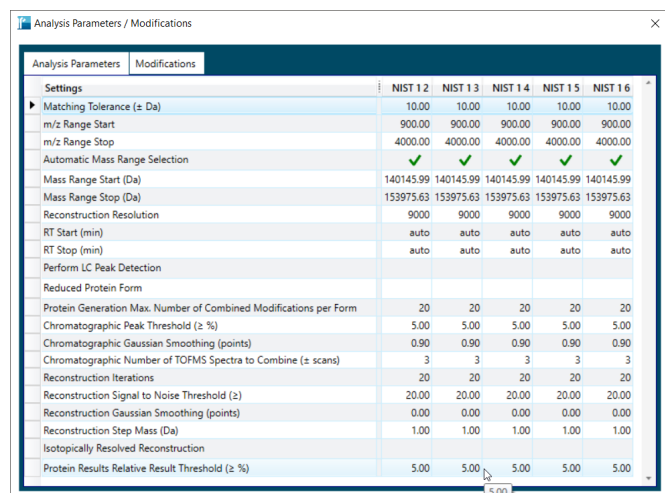
**Figure 3.** Summary of selected proteoforms and relative percentage plot based on reconstructed peak area.



**Figure 4.** Submission of analyses. Multiple samples may be submitted simultaneously, and different processing parameters may be applied to each sample and results stored individually.

After optimization of processing and matching parameters, the defined parameters may be used for ongoing studies. Submission of analyses may be grouped for high throughput processing of data as shown in Figure 4. In addition, if it is required, different processing parameters may be applied to each data file during the same batch submission. After processing, each result is saved separately for comparison within the same review window.

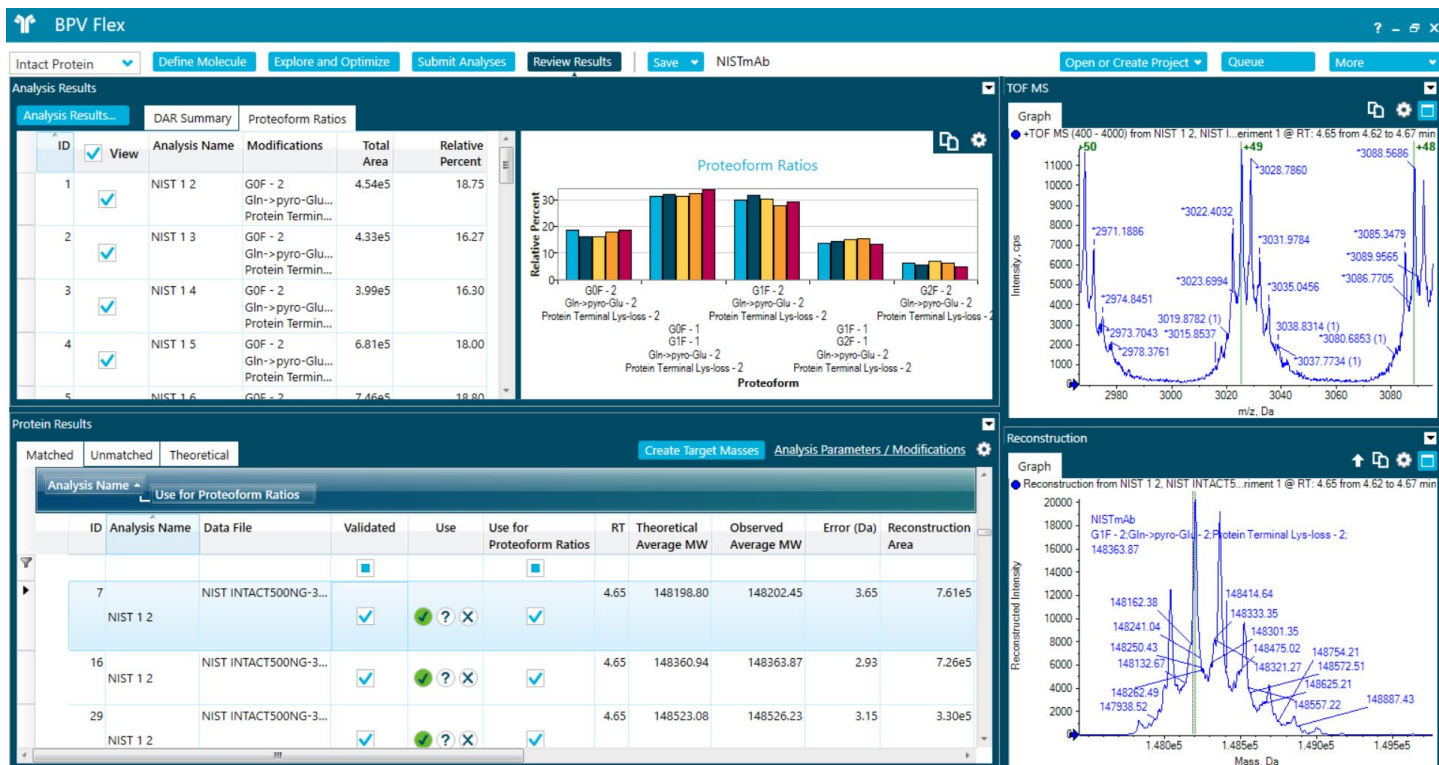
Shown in Figure 6, are the results from processing multiple analyses of NISTmAb. The data were filtered by the analysis name and further by proteoform to expedite review of the processed results. The proteoforms of interest are selected to generate a plot for each glycoform across all of the samples in the study. It is possible to open results from samples processed over time for direct comparison of results without the need to reprocess. In addition, the parameters used for data analysis for each sample, Figure 5, are easily reviewed in a single window to enable correlation of processing parameters to experimental results.



The screenshot shows the 'Analysis Parameters / Modifications' window. It has two tabs: 'Analysis Parameters' and 'Modifications'. The 'Analysis Parameters' tab is active, displaying a table with columns for 'Settings' and five NIST samples (NIST 1.2, NIST 1.3, NIST 1.4, NIST 1.5, NIST 1.6). The table lists various parameters and their values for each sample.

Settings	NIST 1.2	NIST 1.3	NIST 1.4	NIST 1.5	NIST 1.6
Matching Tolerance ( $\pm$ Da)	10.00	10.00	10.00	10.00	10.00
m/z Range Start	900.00	900.00	900.00	900.00	900.00
m/z Range Stop	4000.00	4000.00	4000.00	4000.00	4000.00
Automatic Mass Range Selection	✓	✓	✓	✓	✓
Mass Range Start (Da)	140145.99	140145.99	140145.99	140145.99	140145.99
Mass Range Stop (Da)	153975.63	153975.63	153975.63	153975.63	153975.63
Reconstruction Resolution	9000	9000	9000	9000	9000
RT Start (min)	auto	auto	auto	auto	auto
RT Stop (min)	auto	auto	auto	auto	auto
Perform LC Peak Detection					
Reduced Protein Form					
Protein Generation Max. Number of Combined Modifications per Form	20	20	20	20	20
Chromatographic Peak Threshold ( $\geq$ %)	5.00	5.00	5.00	5.00	5.00
Chromatographic Gaussian Smoothing (points)	0.90	0.90	0.90	0.90	0.90
Chromatographic Number of TOFMS Spectra to Combine ( $\pm$ scans)	3	3	3	3	3
Reconstruction Iterations	20	20	20	20	20
Reconstruction Signal to Noise Threshold ( $\geq$ )	20.00	20.00	20.00	20.00	20.00
Reconstruction Gaussian Smoothing (points)	0.00	0.00	0.00	0.00	0.00
Reconstruction Step Mass (Da)	1.00	1.00	1.00	1.00	1.00
Isotopically Resolved Reconstruction					
Protein Results Relative Result Threshold ( $\geq$ %)	5.00	5.00	5.00	5.00	5.00

**Figure 5.** Rapid review of parameters used for data analysis across samples for expedited comparison.



**Figure 6.** Multiple samples may be submitted simultaneously, and different processing parameters may be applied to each sample and results stored individually.

## Conclusions

- BPV Flex software 1.0.1 provides an efficient solution to refine and optimize processing parameters using an interactive interface
- Expedited assay definition by reuse of molecule information, processing parameters, and review preferences to enable flexible processing and review of studies
- Flexible and intuitive filtering to focus on the most important results
- Data review in BPV Flex software 1.0.1 is easily accomplished using a customizable interface to focus on relevant information
- Track components across studies with automatically generated plots of critical species

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