# Utilization of Intact Reconstruction to Characterize Bi-specific Antibodies

William Resager[1], Nancy Fernandes[2]
Protein Metrics, Boston, MA[1], Lonza Biologics, Portsmouth, NH[2]

## Summary

The aim of this study is to utilize the newly developed Intact Reconstruction workflow to quickly characterize bsAbs, addressing challenges of mispairing chains, varied glycan profiles, and PTMs.

Simultaneous processing of CE-MS in native conditions and tryptic peptide mapping data in one workflow streamlines a qualitative comparative assay.

Overlaying the datasets facilitates proper identification, validation, and annotation assignments in the CE-MS dataset.

The streamlined workflow facilitates expedited visual clues to discern outliers within the purview of both datasets.

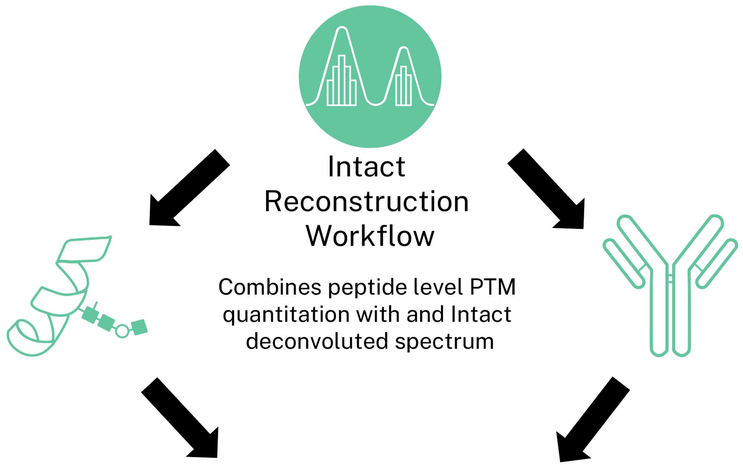
## Introduction

Bi-specific antibodies (bsAbs) represent an important expansion of biotherapeutics. With their ability to target multiple epitopes or antigens, bsAbs are an emerging biotherapeutic to treat rapidly changing and complex diseases such as cancer while maintaining their effectiveness compared to traditional treatments. With this ability comes unique challenges to characterize the final constructs from manufacturing. The propensity for mispairing chains, complex glycan profiles, and additional post translation modifications (PTMs) lead to a large number of similar masses that traditionally require several different approaches to properly characterize the bsAbs. In this study, we utilize intact reconstruction to integrate tryptic digest peptide data with intact mass spectrometry (MS) data using a newly developed workflow to quickly characterize bsAbs.

## Methods

An aliquot of bsAbs was denatured followed by tryptic digest for 195 minutes. The sample was separated on C18 column using a 82 min gradient and injected into aThermo Vanquish liquid chromatography instrument coupled to a Thermo Scientific Exploris 120 mass spectrometer. Intact mass analysis was performed on the same bsAbs using capillary elecrophoresis mass spectrometry (CE-MS) in native conditions. The data was collected using ZipChip® capillary electrophoresis coupled with a Bruker Compact II QTOF mass spectrometer. The data was then processed using an optimized Intact Reconstruction workflow (Protein Metrics) and the peptide data manually validated. The two datasets were then overlaid using the reconstruction feature and the intact charge profile visualized.

## Workflow



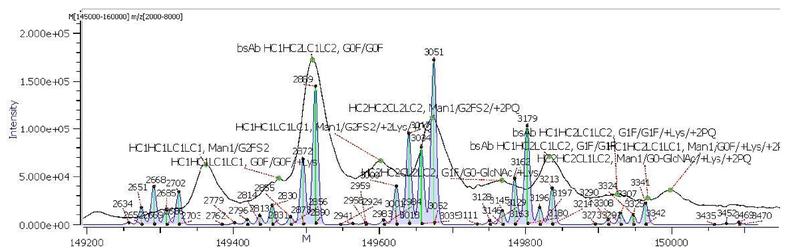




Figure 1: Deconvoluted bsAb Trace with peptide reconstruction overlaid

## Results

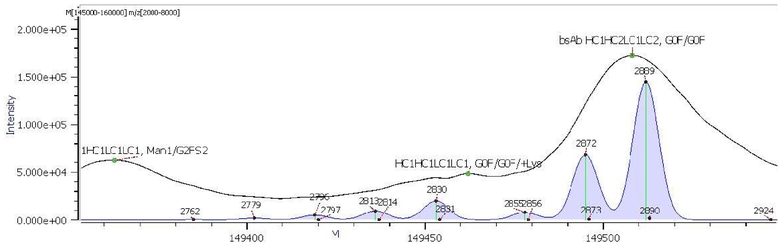



Figure 2: bsAb Reconstruction zoomed M149350-14955

Looking at the figure above the reconstruction can explain the variations in the peak widths and over behaviour of the trace. The two peaks lacking from the reconstruction can be attributed to mispaired bsAb that was not contained within the reconstruction parameters. The high mass shoulder of bsAb G0F/G0F is likely also attributed to a mispaired species that was unresolved within the spectrum.

## Results

The newly developed intact reconstruction workflow is able to process both intact and tryptic peptide data simultaniously. The tools availible within the software then allow for the relative intesities of each identified PTM from the tryptic dataset to be overlaid across the deconvulted intact spectrum to reconstruct the charge profile. From this we were able to properly identify, validated, and adjust annotations of the signals from the Native CE-MS datasets. All basic and base peak signals were validated for confident annotations. Limited ionization efficiency lead to low resolution data and will be improved in future studies. Glycan profiles and PTM combinations are then compared to the theoretical overlay allowing for optimization of workflows going forward.

The Authors declare no conflict of interest. All trademarks belong to Lonza or its affiliates or to their respective third party owners. The information contained herein is believed to be correct and corresponds to the latest state of scientific and technical knowledge. However, no warranty is made, either expressed or implied, regarding its accuracy or the results to be obtained from the use of such information and no warranty is expressed or implied.