

# On-demand Online Reduction of Intact Antibodies for Automated Workflows on SampleStream coupled to a timsTOF

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## Introduction

Online sample processing coupled with mass spectrometry is a powerful method for high throughput analysis of intact proteins.

Adding a chemical process to an MS workflow can be logically challenging, especially when dealing with numerous samples. User-to-user variability is also a consideration which directly impacts data interpretation. Furthermore, some proteins become less stable after chemical processing making point-of-use technologies a necessity.

Here we introduce a new automated workflow for the efficient analysis of proteins featuring an easy-to-use acquisition pipeline that removes user related variability and facilitates on demand experiments for an intact and a reduced measurement from the same vial using 100 ng at a time.



Figure 1. Elute SampleStream. This peripheral can be easily integrated into current mass spectrometry workflows. It can perform various sample preparation steps prior to MS introduction, such as rapid solvent exchange, and/or sample preconcentration. This device was configured to be compatible with a wide range of solvents. Operation is performed all under Hystar, to ensure a seamless workflow transition from sample preparation to mass spectrometry.

## Methods

A standard antibody (148 kDa RM 8671 - NISTmAb, Humanized IgG1k) was prepared with different solvent matrices, 20mM Ammonium Acetate, Phosphate Buffered Saline Solution with 5% Sucrose and 25 mM HEPES buffer + 5% DMSO + 0.5% formic acid. The final target antibody concentration was 0.100 mg/ml. Carbonic anhydrase (26kDa) was used to optimize the timsTOF response at the smaller m/z region. The SampleStream membrane used was 5 kDa MWCO and 30% Methanol + 0.3% formic acid solvent buffer was loaded into the solvent bottle.

SampleStream data were acquired in positive mode operating the instrument (Bruker timsTOF HT) in MS parameters optimized for small and large proteins respectively. Pre-processing of raw data (i.e., spectra extraction from chromatogram regions) was performed in Bruker DataAnalysis software.

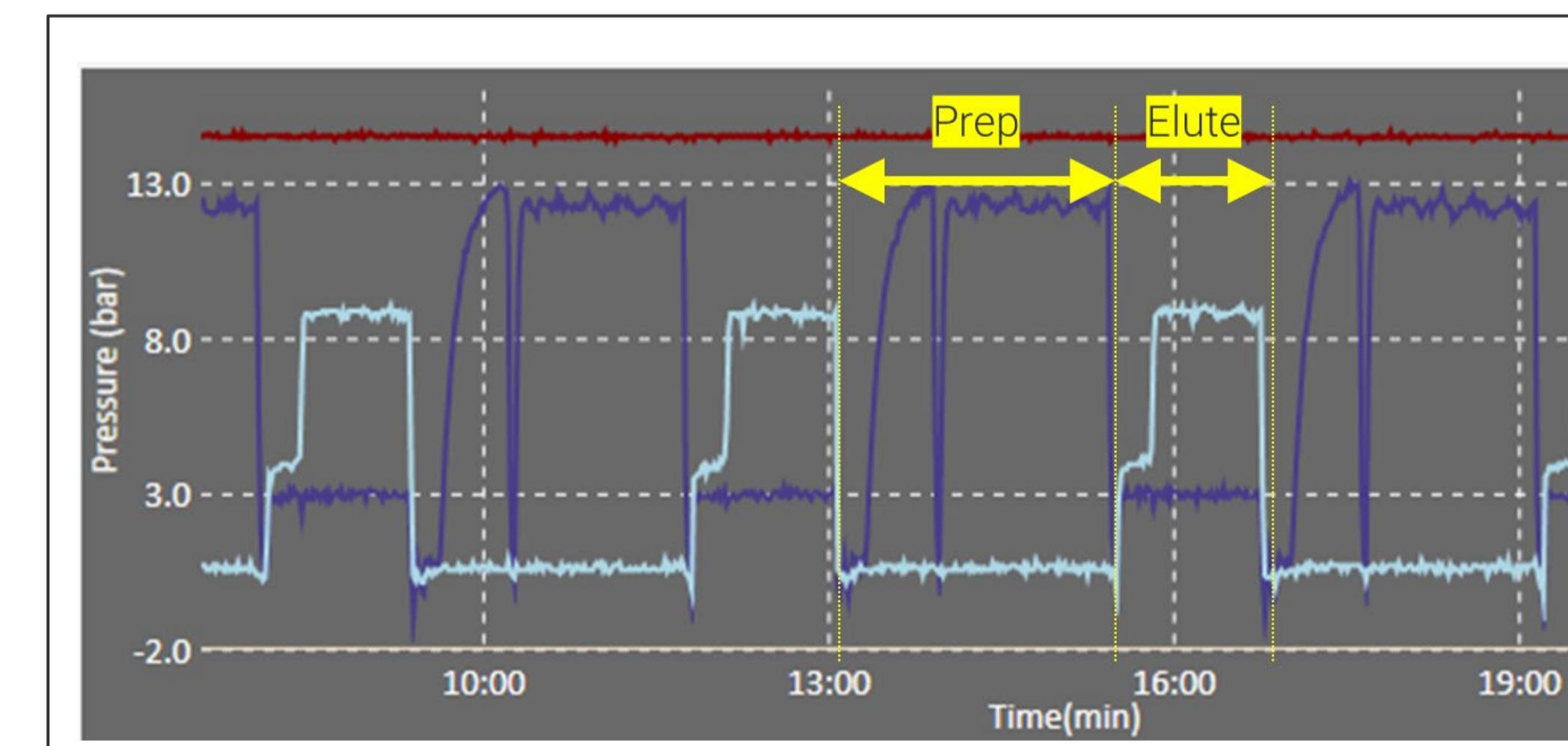


Figure 2. SampleStream workflow timing. Prep time: Injection + Focusing (+ Reaction time). Elute time: Sample is eluted to the mass spectrometer. Flow rates are primarily limited by exerted pressure on membrane.

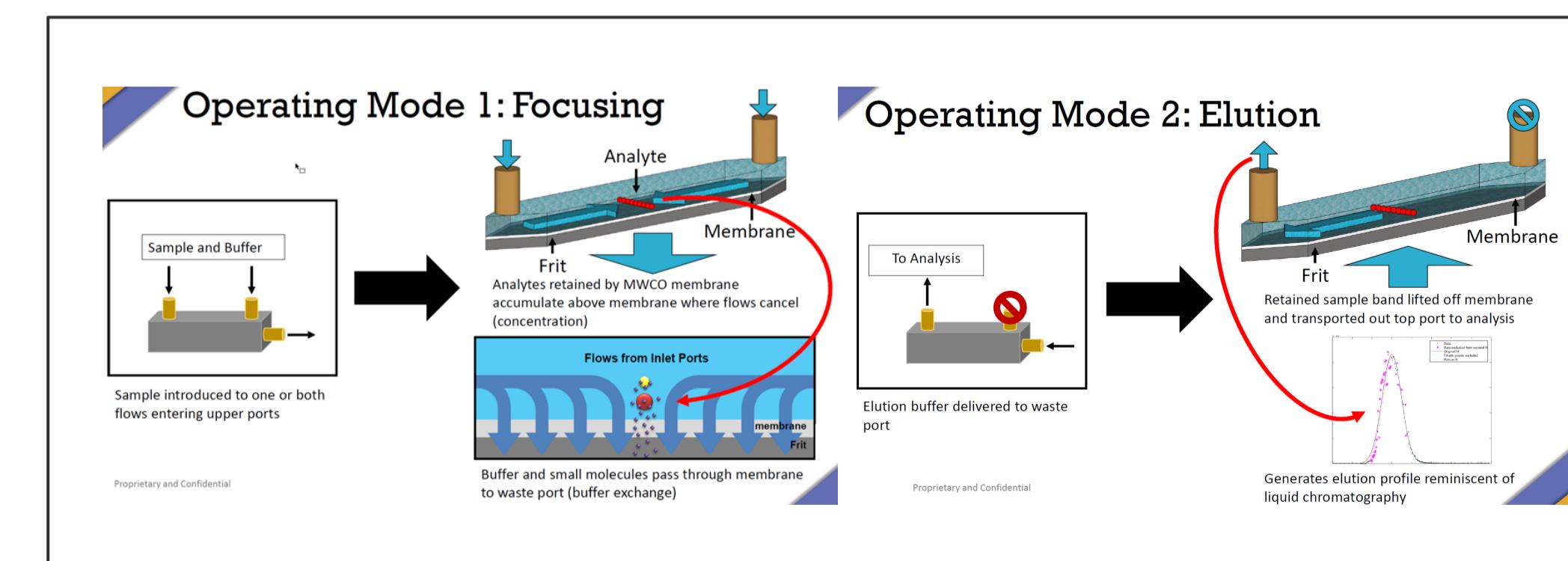


Figure 3. SampleStream workflow explained. Mode 1: Focusing - Analytes are introduced to and retained by MWCO membrane while solvents and small molecules pass through and directed to waste. Mode 2: Elution - Retained sample lifted off membrane and directed to MS.

## Results

For initial MS analysis, data was acquired using a C4 column and Elute HT system to provide a sample reference of the final solutions. Some optimizations were done using carbonic anhydrase to create a method specifically to detect the reduction products. Injection volume was 1  $\mu$ l for sample amount of 100ng on column.

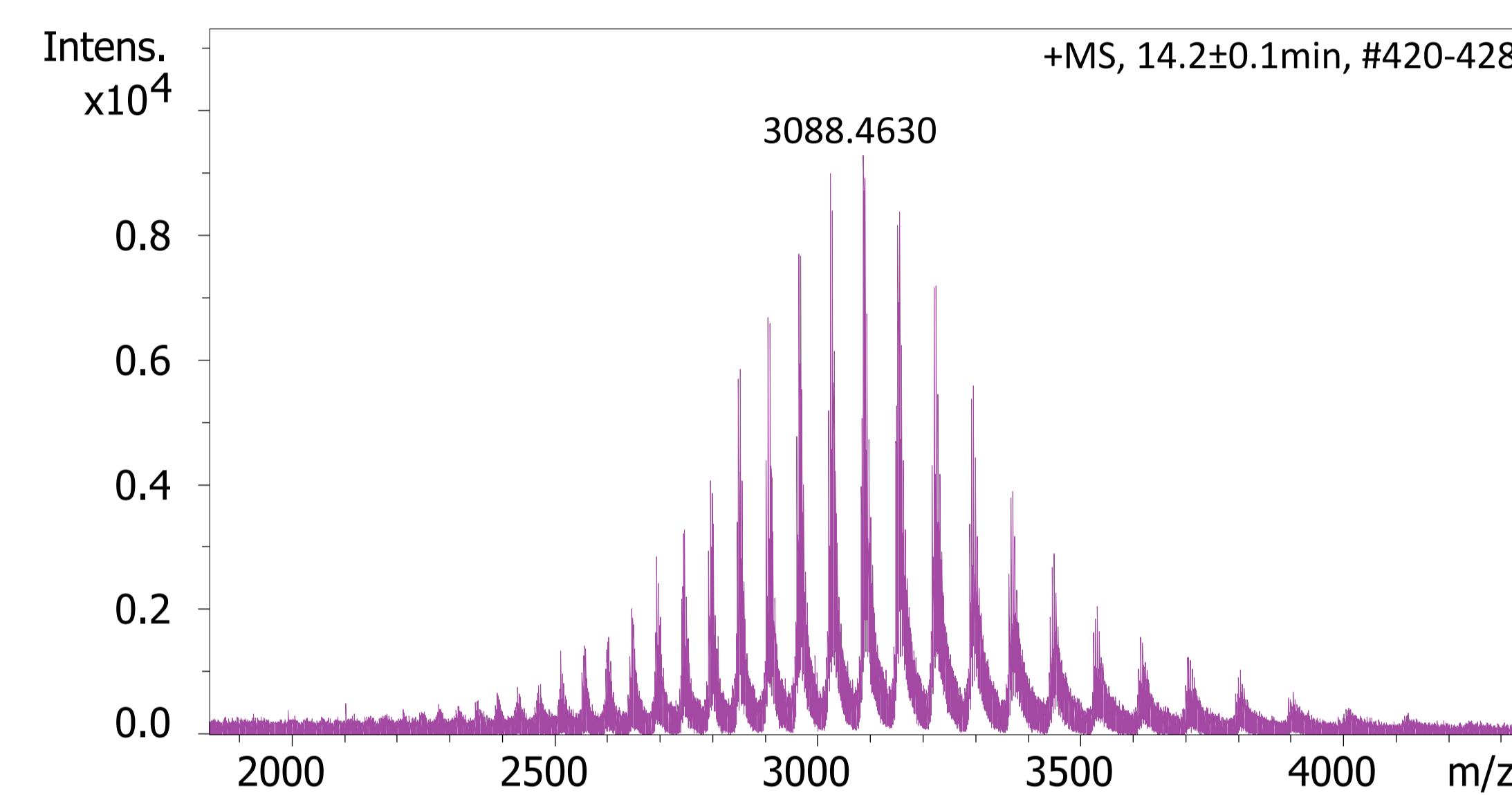


Figure 4. When coupled to the timsTOF HT via the Apollo II ESI source, SampleStream provided efficient sample preparation and automated analysis of intact antibodies. Eluted at 100  $\mu$ L/min after SampleStream cleanup, 100ng NISTmAb injection produced high quality spectra regardless of the initial sample matrix.

### Online Reduction Workflow

Reagent: 50mM TCEP + 7.2M Urea or 50 mM TCEP only

- 20 $\mu$ L + Sample + 20  $\mu$ L – Pre and post sample volumes "Sandwich method"
- 30s Reaction time - wait time with no flow (no under membrane rinse + no counter flow during sample push)
- Analysis time ~3-5 min, depending on reaction and focusing time

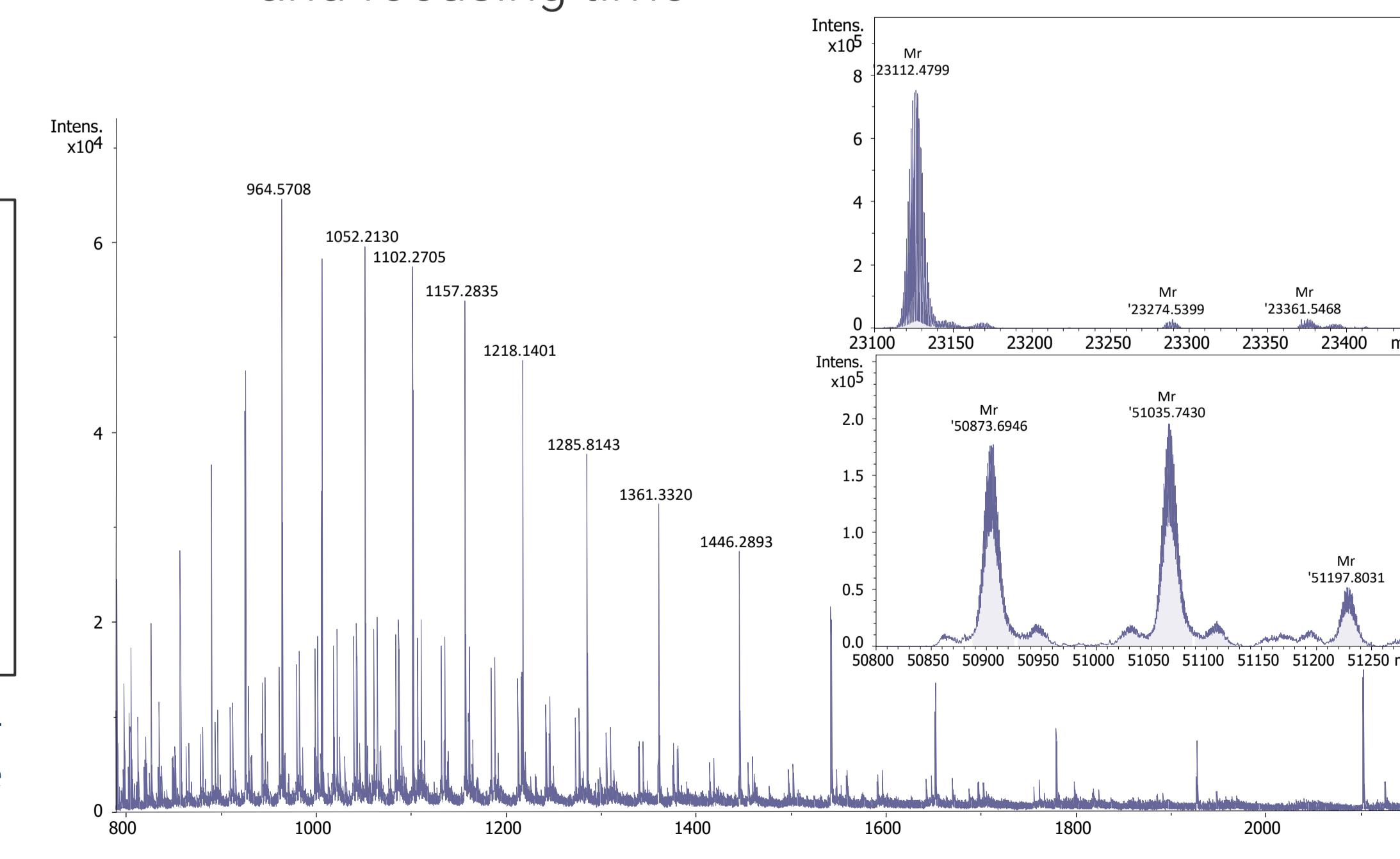


Figure 5. Online reduction NISTmAb. 50mM TCEP with 7.2 M Urea was loaded into a vial and stored in the autosampler along with the samples. The same vial from the intact analysis was sampled. An inset of the deconvoluted data is shown (light and heavy chain).

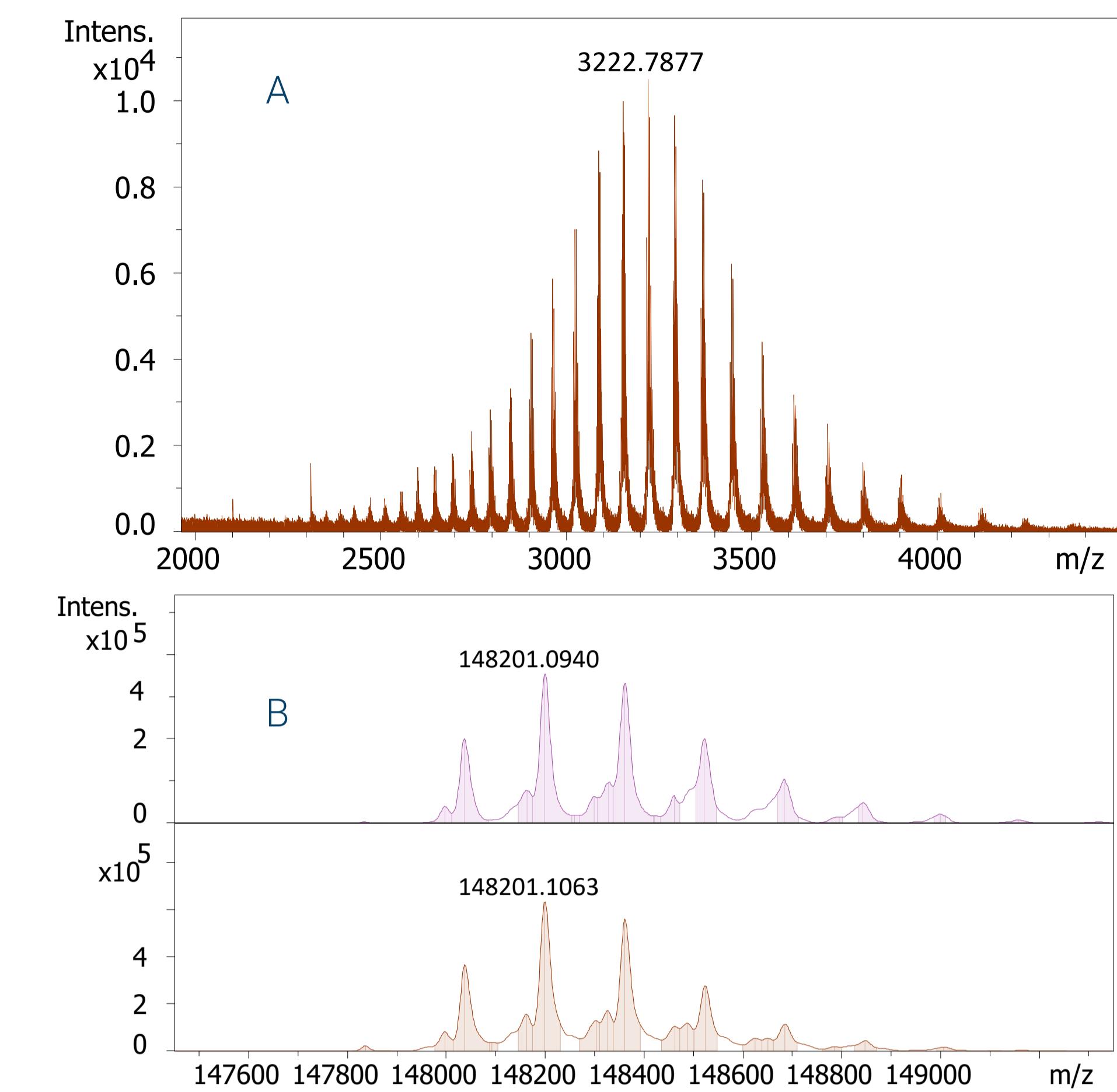


Figure 6. A) NISTmAb injection subsequent to reduction experiment. No reagent carryover, only the intact mAb is observed B) Deconvoluted data comparison between before and after online reduction injection.

## Summary

We have demonstrated an application to expand the capabilities of the SampleStream. Chemical reduction, a process that is embedded in most -omics workflows, can be automated with ease and reproducibility in this platform technology.

Online on-demand capabilities allows convenience and flexibility of performing experiments, especially when sample limited. Carryover from reduction reagents are non-existent.

## Conclusion

- High-throughput analysis interfaced with the Bruker timsTOF systems
- Time-consuming processes such as chemical reduction is performed and integrated with solvent exchange.
- On demand process that performs intact and reduced experiments from the same sample vial, 100 ng at a time.

## Point-of-use sample preparation for MS