



# A Fully Integrated and Automated Platform for Screening Biologics

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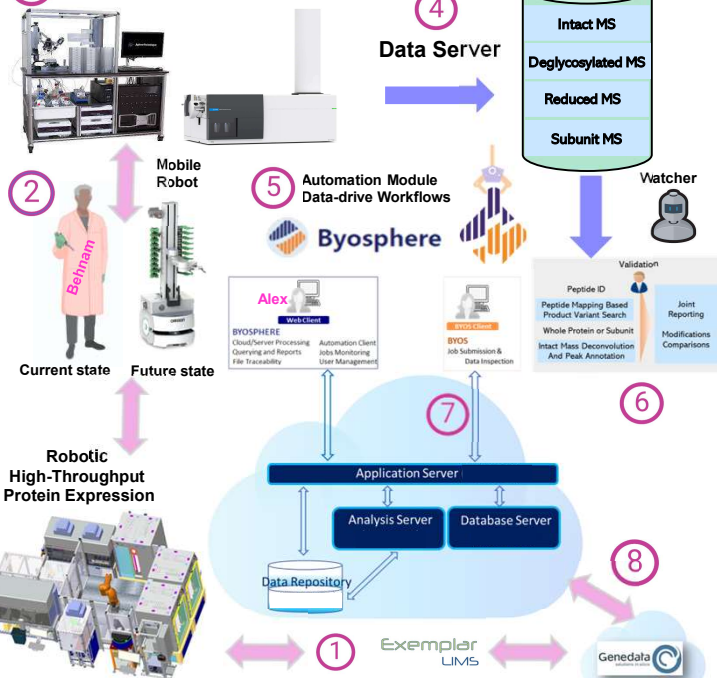


## Introduction

High Throughput (HT) Mass spectrometry (MS) has been indispensable in the discovery of small molecules, peptide/mini protein drug modalities to provide accurate molecular masses with very high accuracy and speed. HT ESI-MS of antibodies was recently demonstrated by Sawyer et al., PNAS, 2020, 117, 9851-9856. Motivated by this work, we have developed a complete end-to-end (E2E) pipeline coupling RapidFire-MS (RF-MS) data acquisition with real-time informatics and data aggregation for biologics. As a result, we enable the integration of production, analytics, and informatics to screen large panels of biologics, e.g.,  $>10^3$ - $10^4$  molecules by streamlining hardware and data automation. We demonstrate the analytical figures of merit of how pipeline delivery of biologics compares to traditional analytical and off-the-bench informatics solutions.

## Methods

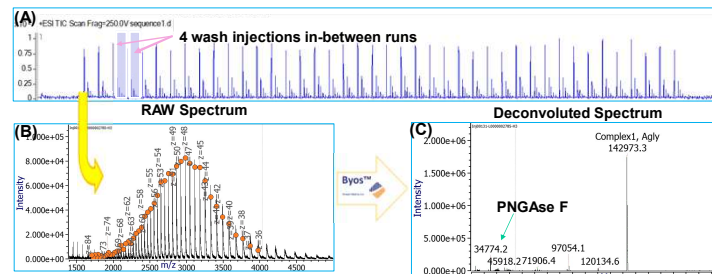
### 3 RapidFire-MS Acquisition



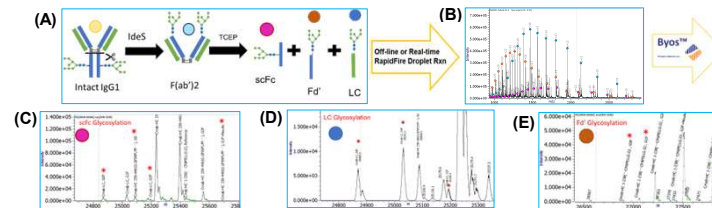
### E2E Automated Sample Preparation, Acquisition and Data Analysis

- Figure 1.** The structure of the RapidFire automation High-Throughput platform for Biologics Screening
1. LIMS system supports the retrieval of samples
  2. Automated protein A purification using MARS robotics and robotic hand-off (Future state)
  3. RapidFire-MS acquisition
  4. Data servers storing data
  5. Automated data sweep to Byosphere platform with fully integrated intact MS Data-driven workflows
  6. Automated export of results and joint reporting of modifications
  - 7&8. Aggregate data with molecule information to build 'In-house' knowledge base (also auto QC)

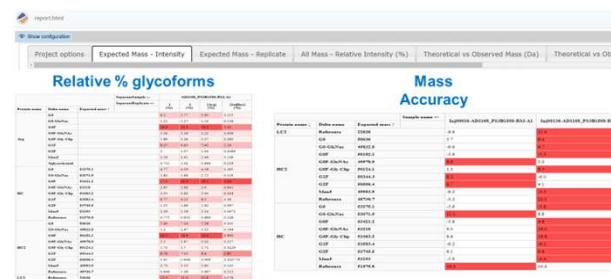
## Results and Discussion



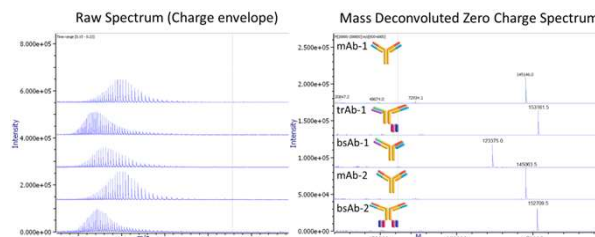
**Figure 2.** The RF-MS system can be applied for a typical large molecule (e.g., antibody) analysis. (A) The system enabling data to acquisition rates as fast as 5 seconds per sample. The sample IDs and locations on the well are matched using metadata files. (B) High quality raw MS data were acquired with high-throughput. (C) Accurate mass was deconvoluted with Byos intact mass deconvolution algorithm.



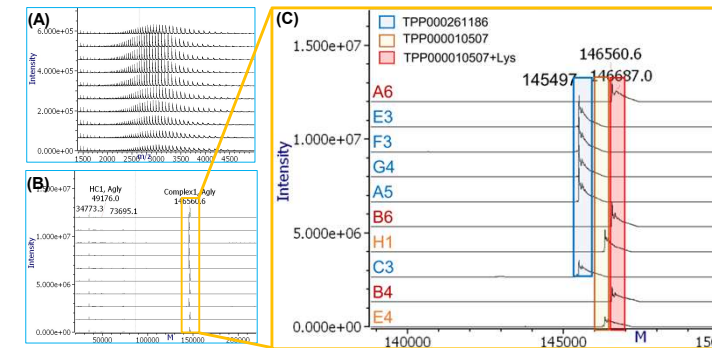
**Figure 3.** Rapid subunit glycoforms analysis via RapidFire. (A) Diagram of subunit digestion and fragments (B) High quality raw MS data were acquired with high-throughput. (C-E) Accurate masses of each fragment were deconvoluted.



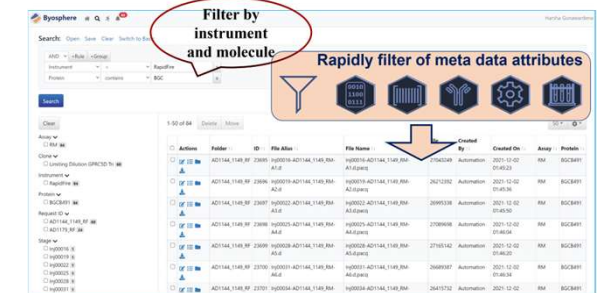
**Figure 4.** Web-based customizable reports showing relative % of glycoforms and the mass accuracy



**Figure 5.** RF-MS spectra of multiple antibody modalities run in a batch after PNGase F deglycosylation



**Figure 6.** RF-MS Batch processing of 96-well plate with masses close to the target mass. (A) The raw data mass spectra of the ten samples. (B) The deconvoluted masses from panel A. (C) The zoom-in image of the ten masses shown the two samples with target mass, three samples with unprocessed Lysin, and five samples with a mass of another target molecule (re-annotation).



**Figure 7.** Byosphere advanced search capabilities based on meta data attributes

## Summary

- Large panels of biologics generated in HT-Protein Expression are screened immediately using a RapidFire coupled with a quadrupole time-of-flight mass spectrometer (RF-MS).
  - This E2E workflow to identify hundreds of large molecules in a day with minimal human intervention
- The acquisition is hands-off and significantly faster compared to conventional LC-MS approaches.
  - Comparable data quality to LC-MS was obtained.
- The well-designed reporting system shows a comprehensive attributes of biologic molecules
  - Enabling the team get an early insight in the quality of biologics in real-time.
- Raw MS data and associated meta data are swept to an automation module in the Byosphere that resides in the J&J cloud server.
  - Advanced querying capabilities and quality control of data allows triaging voluminous data sets.

The authors declare no competing financial interest.