



Introduction

High Throughput (HT) Mass spectrometry (MS) has been indispensable to the discovery of small molecules, peptide/mini protein drug modalities, providing 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. We have successfully deployed this E2E workflow to identify hundreds of large molecules in a day with minimal human intervention enabling unprecedented insight into the quality of biologics in real-time at HT screening scale. As a result, we enable the integration of production, analytics, and informatics to screen large panels of biologics, e.g., >103-104 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

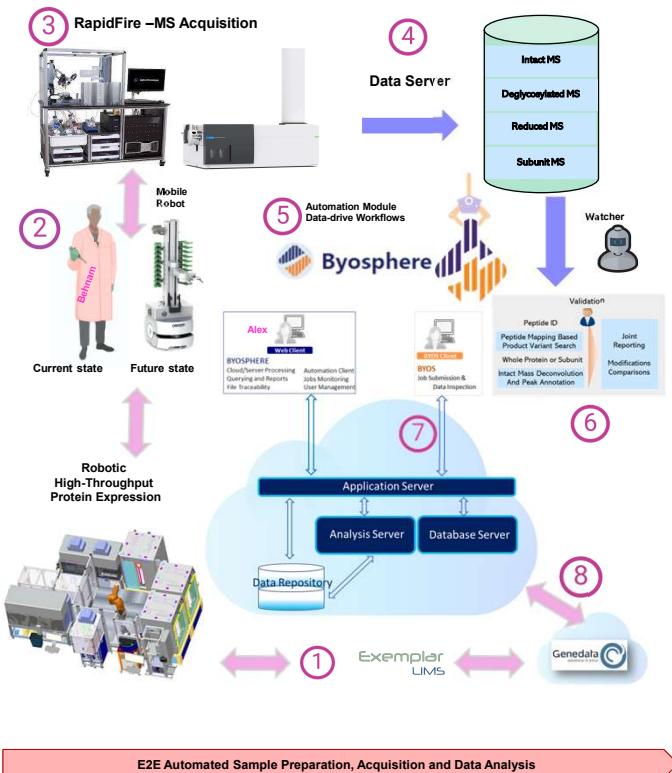


Figure 1. The structure of the RapidFire automation High-Throughput platform for Biologics Screening

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

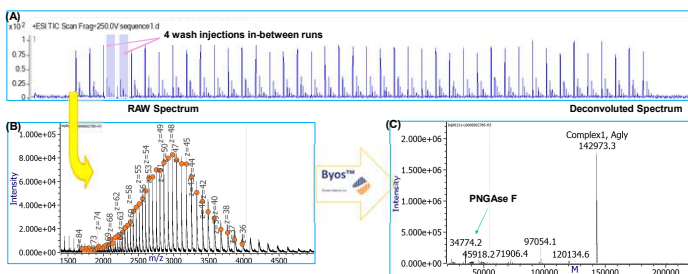


Figure 2. The RF-MS system can be applied for a typical large molecule (e.g., antibody) analysis. (A) The system enabling data to acquisition rates as fast as 5 seconds per sample. The sample IDs and locations on the well are matched using metadata files. (B) High quality raw MS data were acquired with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

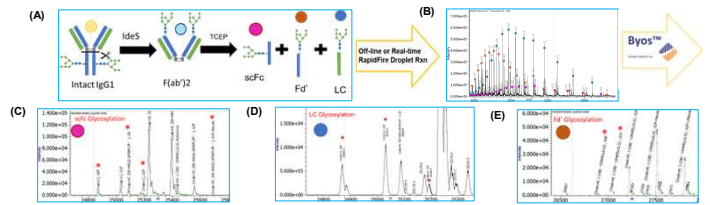


Figure 3. Rapid subunit glycoforms analysis via RapidFire. (A) Diagram of subunit digestion and fragments (B) High quality raw MS data were acquired with high-throughput. (C-E) Accurate masses of each fragment were deconvoluted.

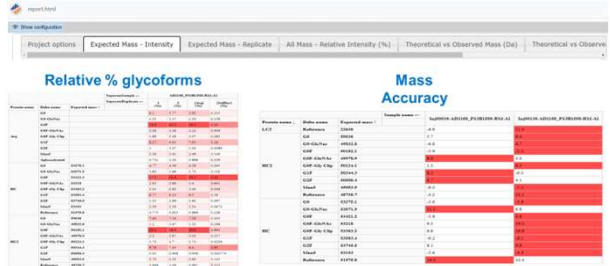


Figure 4. Web-based customizable reports showing the relative percentage of glycoforms and the mass accuracy

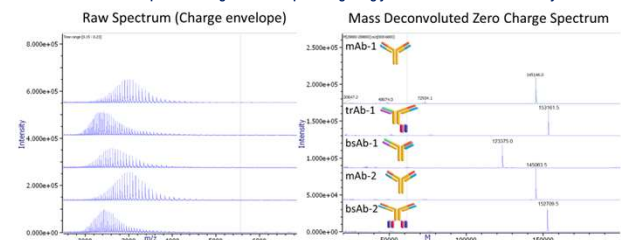


Figure 5. RF-MS spectra of multiple antibody modalities run in a batch after PNGase F deglycosylation

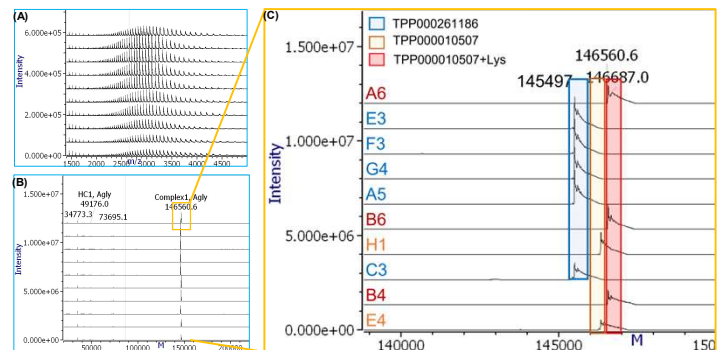


Figure 6. RF-MS Batch processing of 96-well plate with masses close to the target mass. (A) The raw data mass spectra of the ten samples. (B) The deconvoluted masses from panel A. (C) The zoom-in image of the ten masses shown the two samples with target mass, three samples with unprocessed Lysin, and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

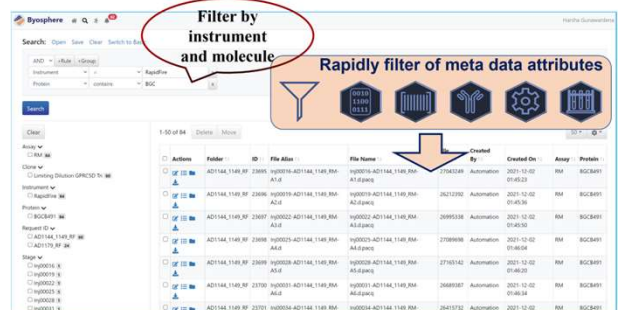


Figure 7. Byosphere advanced search capabilities based on meta data attributes

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.*