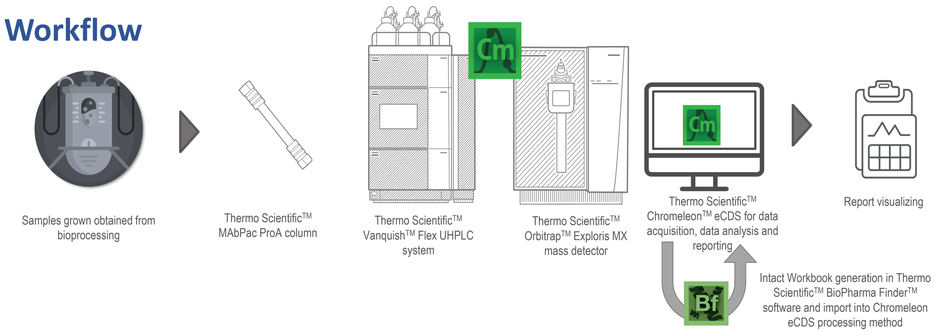
At-line monoclonal antibody analysis using affinity-chromatography with mass spectrometry detection and fully compliant data acquisition and processing

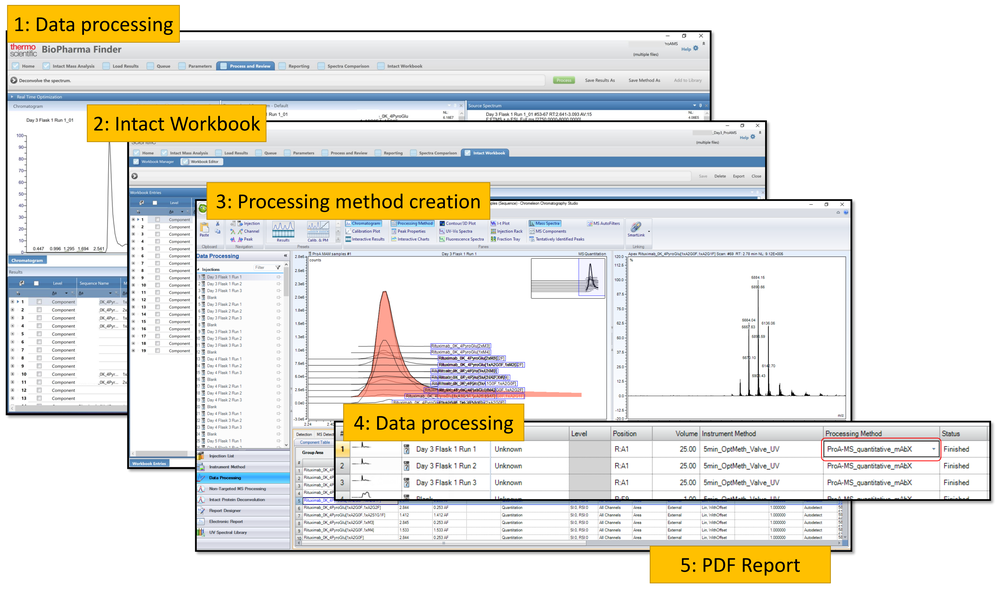
Sara Carillo[1], Craig Jakes[1,5], Florian Fuessl[1], Jennifer Sutton[2], Julia Kraegenbring[3], Catharina Crone[3], Kai Scheffler[4], Jonathan Bones[1,5]

[1]National Institute for Bioprocessing Research and Training (NIBRT), Blackrock, Ireland; [2]Thermo Fisher Scientific, San Jose, CA;
[3]Thermo Fisher Scientific, Bremen, Germany; [4]Thermo Fisher Scientific, Germering, Germany; [5]University College Dublin, Dublin, Ireland

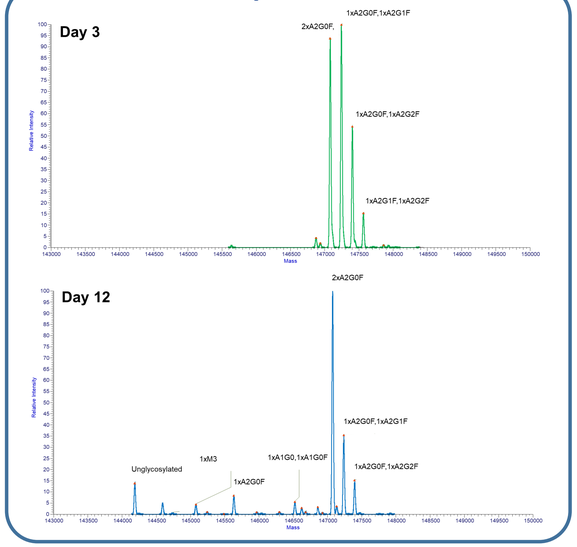
- **Background:** Monoclonal antibody (mAb) drug products are constituted by a mixture of proteoforms whose relative abundances are influenced not only by the expression system, but also from upstream and downstream processing steps. There is an increasing need to closely monitor these proteoforms, not only from early stages of product development to speed up clonal selection, but also to monitor production batches during expression and purification to be able to fine tune proteoforms quality and quantity in the final product. Here, we describe the use of affinity chromatography directly coupled with the Thermo Scientific™ Orbitrap Exploris™ MX mass detector to monitor trends of IgG1 proteoforms during a bioreactor campaign using fully compliant data acquisition and processing (intact Multi-Attribute Method – iMAM).
- **Methodology:** Samples derived from a bioreactor campaign were aseptically collected, clarified from cell debris and directly analysed on a Thermo Scientific™ Vanquish™ Flex UHPLC hyphenated with a full-MS only Thermo Scientific Orbitrap Exploris MX system. Thermo Scientific™ Chromeleon™ CDS software was used to ensure that the platform was compliant for data acquisition, storage and processing. Samples were analysed using a Thermo Scientific™ MAbPAc™ Protein A column using a step gradient of 50 mM ammonium acetate (pH 7.0) and acetic acid at pH 2.5. Online processing of the data acquired was performed using Chromeleon CDS software using a workbook containing the proteoforms to be monitored, to generate a report of the proteoform trends and to evaluate acceptance criteria.

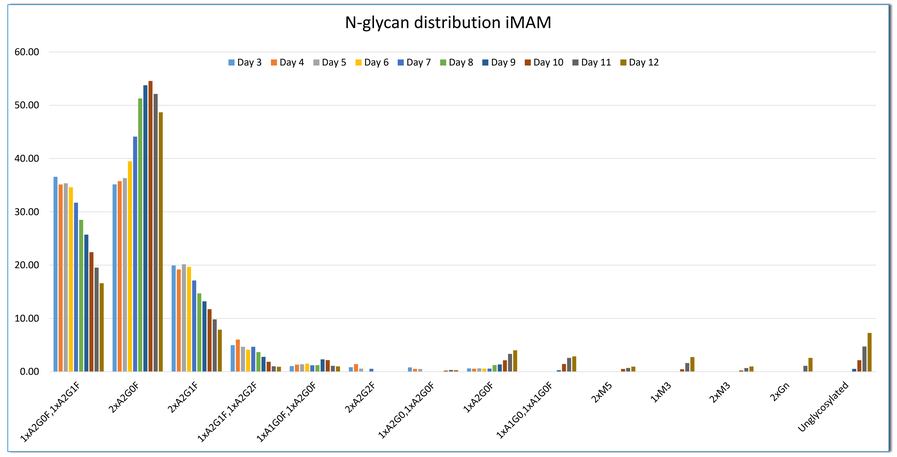
Workflow

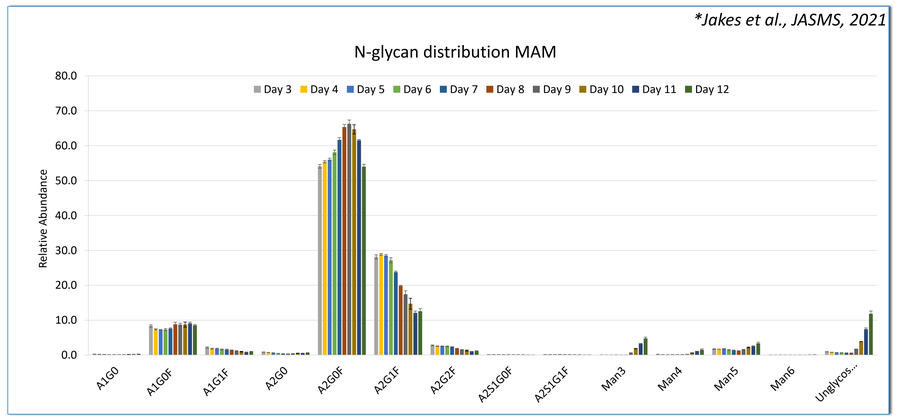




Deconvoluted Spectra







Results: The report obtained after the analysis performed at-line from day 3 to day 12 shows mass accuracy and reproducibility of the data was maintained throughout the experiment length. Deconvoluted spectra showed considerable change in the N-glycans with increase of unglycosylated species as well as high mannose N-glycans. iMAM workflow allowed to monitor this trends in a similar way to what was obtained through a standard MAM approach using peptide mapping [2]. Differences in the relative abundance can be explained as iMAM workflow allows the evaluation of N-glycan pair rather than the single N-glycan amount.

Conclusions: In the present study, 5 minutes long ProA-MS workflow hyphenated with the Thermo Scientific Orbitrap Exploris MX was used to monitor at-line product quality attributes during a bioreactor campaign. A processing method was created using Chromeleon CDS software and used to calculate proteoforms variability during cellular growth. Main PQAs to change during the campaign were N-glycans that were accurately monitored, similar to what was performed with a standard MAM approach that involves sample purification and lengthy sample preparation and analysis.

Acknowledgment: The authors gratefully acknowledge Thermo Fisher Scientific for instrument access and support.

References: [1] Jakes et al. Anal. Chem. 2021, 93, 40, 13505–13512
[2] Jakes et al. J. Am. Soc. Mass Spectrom. 2021, 32, 8, 1998-2012