Rapid Characterization of Unpurified Biotherapeutics through Online Buffer Exchange

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Introduction

Development of machine learning and hardware automation through biologics development necessitated the development of higher throughput characterization methods. Characterization by mass spectrometry generally requires highly purified samples typically performed through a Protein A-based affinity purification for IgGs . Though the purification is effective, it requires approximately 4 hours, and specialized labware before LC-MS analysis. Here, we demonstrate the utility of online buffer exchange (OBE) for the characterization of multispecific anitbodies sampled directly from crude supernatant samples taken from a bioreactor. Samples can be analyzed at a rate of *3 minutes per sample* without the need for any sample preparation in a fully automated fashion.

Methods

OBE native MS data was acquired on a Thermo Scientific Q Exactive UHMR hybrid quadrupole -Orbitrap MS system to reveal, ID, and quantify intact antibodies and free chains. An OBE column (2.1 mm ID, 3 µm, 80 Å, 5 cm) was equilibrated with 200 mM ammonium acetate to analyze these samples with a 0.5-1.0 µg load. Importantly, a divert valve was used post-OBE column to divert eluents not containing the biotherapeutic of interest to waste to prevent fouling of the MS. Samples were expressed in-house and aliquots were removed from bioreactors at daily time points for analysis. The MS data were analyzed automatically after each run using novel deconvolution, clustering, and data processing software.

A multispecific antibody was expressed in AMRBR bioreactors in-house over 16 days. Daily aliquots were removed from the bioreactors and centrifuged to remove the cell pellets. Remaining supernatants were analyzed directly by OBE native MS.

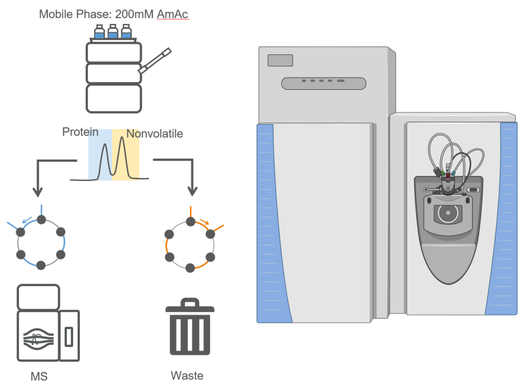

Figure 1. OBE was performed using a Thermo Vanquish LC where a divert valve was utilized to direct only the therapeutic of interest towards the MS for mass analysis. A Thermo Scientific Q Exactive UHMR was used for mass analysis with careful tuning to detect aggregates in the samples.

Intact Mass Analysis

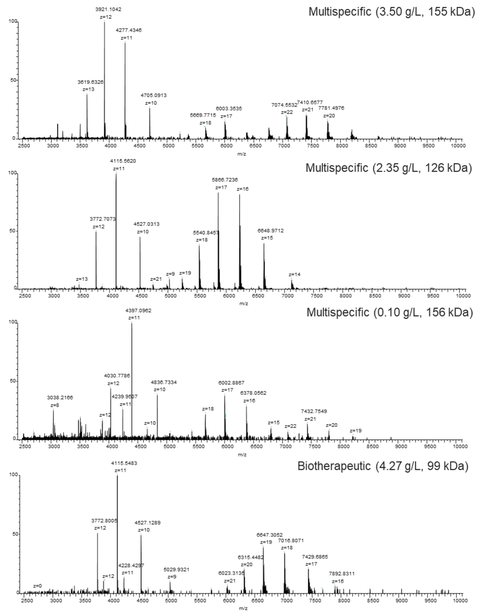

Figure 2. Four representative mass spectra acquired by OBE native MS. The four samples varied in MW from 99 kDa to 156 kDa and varied in concentration between 0.1 g/L and 4.3 g/L. Charge state distributions at lower m/z are excess light chain, heavy chain, or half mAb depending upon the specific sample that are typically purified out using ProA affinity purification.

OBE was shown to be a molecule agnostic method for molecular weight confirmation without any requirements for purification nor method development. Here, four different molecular constructs were taken directly from cellular supernatants with varying expression levels (0.1-4.3 g/L) and successfully confirmed the molecular identity of the therapeutic. Moreover, OBE native MS provides a method wherein none of the excess chains nor half mAbs are purified out of analyzed solutions lending insight in the expression levels of all chains.

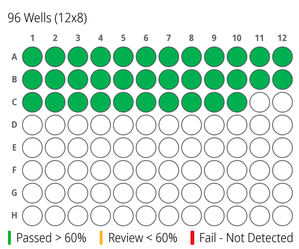

Figure 3. OptiMSe can provide automated data processing for molecular weight confirmation for targeted therapeutics or OBE native MS acquired data.

Bioreactor Time Course Study

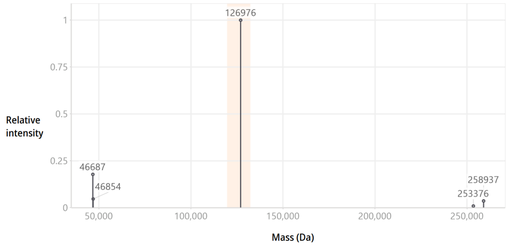

Figure 4. Representative deconvoluted mass spectrum of the biotherapeutic at day 4 as processed automatically by OptiMSe. Highlighted at 127 kDa shows the therapeutic of interest.

Aliquots from AMBR bioreactor expressions of a multispecific antibody were taken at daily timepoints across a 16 day expression cycle in duplicate. Samples from the bioreactor were centrifuged to separate the cell pellets and the unpurified supernatants were analyzed directly by OBE native MS in just three minutes. Four dominant species were detected in the sample across the timepoints: the desired monomer, a light chain (LC) dimer, a half mAb (LC+HC), and a dimer of the therapeutic. Early in the bioreactor run, before 4 days, the cells appear to overexpress light chain resulting in high levels of excess light chain dimer. By day 5, the monomer abundance increases with an inverse correlation to light chain dimer. Moreover, as more monomer is expressed, the presence of aggregates begins at day 4 and starkly increased rapidly on day 8. Monomers and aggregates dominate the bioreactors by day 10.

Ultimately, this method can provide near real-time feedback to bioreactor runs to not only confirm molecular weight, but to also provide insight into critical product quality attributes that other MS-based methods are unable to provide.

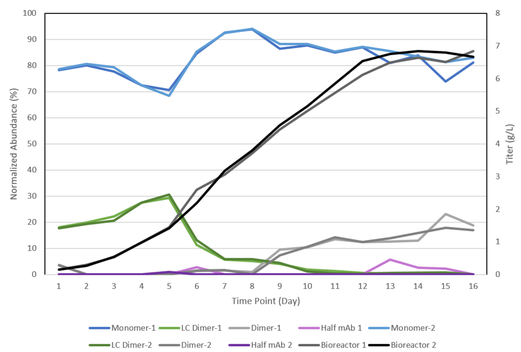

Figure 5. Normalized abundances across 16 days of the four detected species related to the biotherapeutic by OBE native MS: monomer, LC dimer, dimer, and half mAb (LC+HC).

OptiMSe Reporting

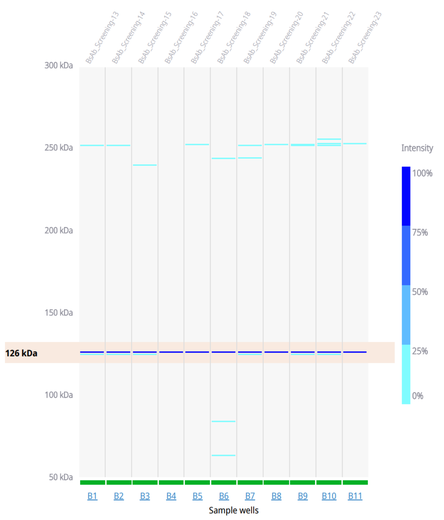

Figure 6. In addition to deconvoluted MS and MW confirmation, OptiMSe generates MW ladders similar to gels for reports to scientists unfamiliar with MS data.

Summary

Online buffer exchange native mass spectrometry provides a powerful and rapid technique to analyze unpurified biotherapeutics directly from cellular supernatants. The technique was shown to work for a variety of bio therapeutics: monoclonal antibodies, multispecific antibodies, and novel biotherapeutics, without the need for protein purification before analysis. Moreover, no additional method development was required despite the wide range of molecular weights and concentrations.

OBE native MS was also utilized to assess the product quality of a multispecific antibody during a 16 day bioreactor expression. It successfully detected noncovalent multimers and showed a steady increase of aggregation over time that correlates with increased titer.

This technique provides a rapid method to characterize biotherapeutics using intact MS and can be expanded to analyze for a variety of product quality attributes.

References

VanAernum ZL, Busch F, Jones BJ, Jia M, Chen Z, Boyken SE, Sahasrabuddhe A, Baker D, Wysocki VH. Rapid online buffer exchange for screening of proteins, protein complexes and cell lysates by native mass spectrometry. Nat Protoc. 2020 Mar;15(3):1132-1157.