Automated High-throughput Online Native MS Screening for Proteins and Protein Complexes

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ABSTRACT
Application of an online automated OBE native MS (Online Buffer Exchange) workflow to quickly screen for sample integrity. Additionally, optimal buffer conditions were determined for the Mem-NB complex using this workflow

INTRODUCTION
As the automation and achievable resolution of cryo-electron microscopes is rapidly improving, the critical step is now in preparing good grids, which require stable and homogeneous sample. Typical characterization methods like SEC can be hard to interpret especially for membrane proteins due to the heterogeneity introduced by the membrane mimics required for stabilization of the membrane protein. Native mass spectrometry (nMS) has become a prominent tool for MS based structural biology for its ability to preserve intact protein complexes and post-translational structure and can provide valuable information for downstream analysis such as Cryo-EM. Here we present a fully automated native mass spectrometry-based workflow, designed for non-experts, to screen suitable and membrane proteins for stability, homogeneity and composition

MATERIALS AND METHODS
Standard proteins and complexes such as Alcohol Dehydrogenase, Beta-Galactosidase, Prokaryotic Kinase, Prokaryotic Kinase, GniEL were injected onto the Thermo Scientific™ NanoES LC offline buffer exchange column (Fig 1); for sample desalting and buffer exchange into mass spectrometry compatible buffers such as 100 mM Ammonium Acetate (isocratic elution) established by a Thermo Scientific™ VersaPak™ UHPLC (Fig 1). The NanoPac OBE column was directly interfaced with either a Thermo Scientific™ Exactive™ or Thermo Scientific™ Orbitrap™ Q Exactive™ UHMR Fig 1 via the HESI II ion source. Data acquisition, analysis, and result reporting were automated on the-fly by Thermo Scientific™ Xcalibur™ and OptiMS software. Briefly, optimized MS acquisition and data analysis settings are assigned by OptiMS based on user input sample information.

ONLINE BUFFER EXCHANGE Workflow Components

Online Buffer Exchange

Figure 2. OBE Performance

Automated Data Analysis

Figure 3 shows the schematic overview of the fully automated and to end OBE n-MS protein screening workflow. Briefly:
1. Sample Input: The user enters sample information such as target molecular weight, experimental conditions and pass/fail threshold via GUI. The sample tray is then filled according to the virtual design. The sample information is then acquired by the local OptiMS software. The user loads the sample tray into the LC and then uses the OptiMS software to convert the sample information into an Xcalibur run queue and simultaneously stores the parameters for data analysis.
2. Separation & Detection: Simply open the generated run queue and start the run. OptiMS selects the appropriate methods based on the sample information.
3. Process/Report: Once the initial raw file is acquired the data analysis begins running in the background. After the first run is acquired and analyzed, the report generation is triggered (Figures 4 & 5). Finally, the results are archived locally and optionally uploaded to the Athena Imaging Management Software to provide screening results directly to the structural/biological prepping grids for CryoEM analysis.

HIGH THROUGHPUT SCREENING

Figure 4. OptiMS report – Overview and Gel Plot

ONLINE MEMBRANE PROTEIN SCREENING

Figure 5. Mem-Nanobody complex buffer screening

CONCLUSIONS
The OptiMS workflow automates data analysis and filters out poor quality samples:
• Use of automated LC/MS sample introduction and smart parameters lead to high fidelity results.
• High throughput analysis allows for more experimental space to be covered during sample preparation (including buffer selection).

REFERENCES

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Figure 6 shows successful screening of a particularly challenging sample. This complex has a low binding constant and required not only buffer optimization but also increased ratio of membrane to Melb™.