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

Mick Greer[1], Scott Kronewitter[2], Paul Gazis[3], Ping Yip[2], Weijing Liu[3], Rosa Viner[3], Olufemi Adeyemi [4], Albert Konijnenberg[4] Thermo Fisher Scientific, [1]AustinTX, [2]Cambridge MA, [3]San Jose, CA,[4]Eindhoven NL

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 MelB-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 mimetics 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 protein tertiary 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 soluble and membrane proteins for stability, homogeneity and composition

MATERIALS AND METHODS

Standard proteins and complexes such as Alcohol Dehydrogenase, Beta-Galactosidase, Pyruvate Kinase, Pyruvate Kinase, GroEL were injected onto the Thermo Scientific™ NativePAC online 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™ Vanquish™ UHPLC. **Fig 1**) The NativePac OBE column was directly interfaced with either a Thermo Scientific™ Exploris™ 240 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 via Thermo Scientific™ Xcalibur™ and OptiMSe software. Briefly, optimized MS acquisition and data analysis settings are assigned by OptiMSe based on user input sample information.

Figure 1. Native MS-OBE Screening Workflow Components

Online Buffer Exchange
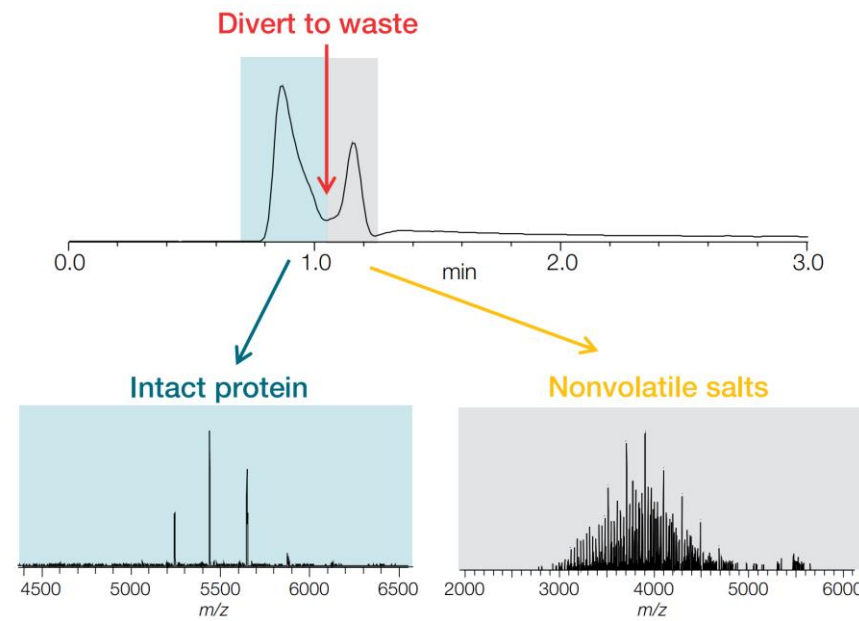
Figure 2. OBE Performance

Figure 2 demonstrates the application of OBE to rapidly and effectively exchange proteins into MS compatible buffers while retaining native conditions. Note the protein or complex is only removed from its original buffer for approximately 30 seconds (or less) prior to MS detection. For sensitive samples OBE may be the ideal method to rapidly buffer exchange while retaining native condition. Moreover, this column enables fast runs requiring only 3 min (or up to 100 sample in 7 hours.)

Automated Data Analysis

Figure 3 shows the schematic overview of the fully automated end 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 exported to the local OptiMSe software. The user loads the sample tray into the LC and then uses the OptiMSe 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. OptiMSe 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 final 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 biologist preparing grids for CryoEM analysis.

Figure 3. Automated Screening Workflow
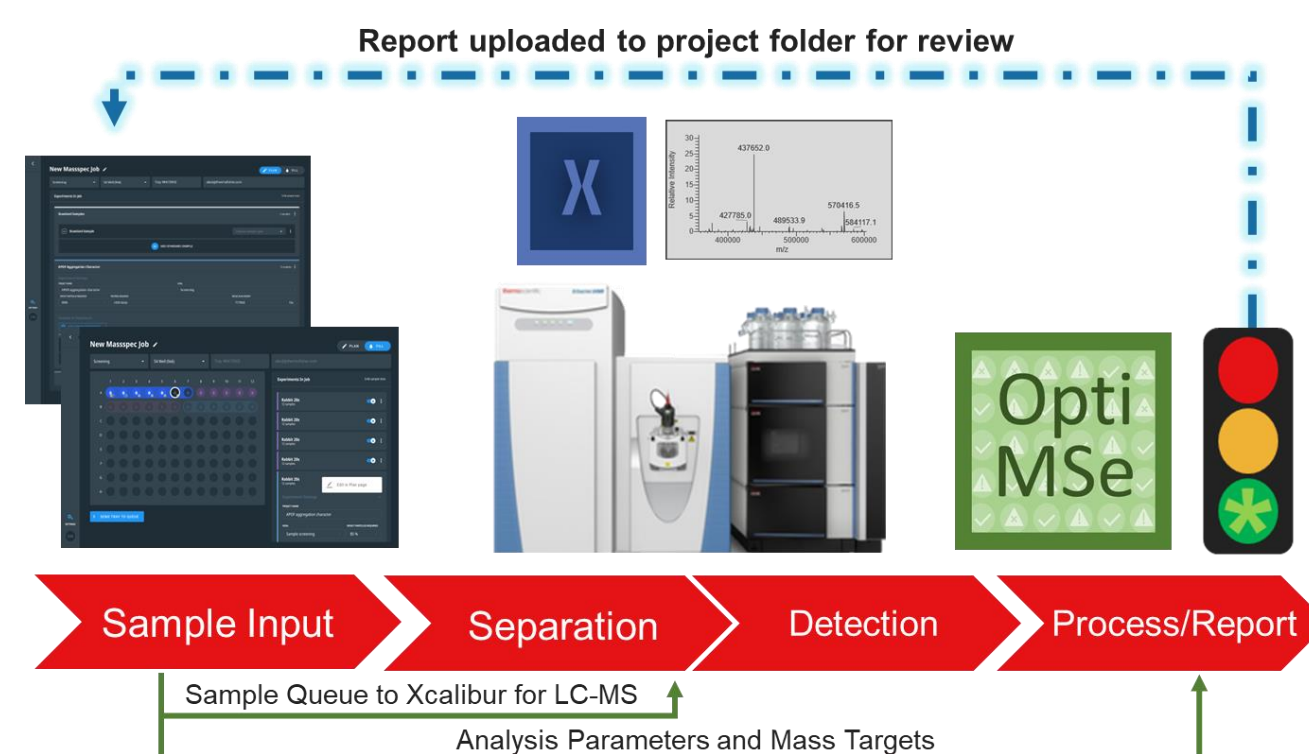
HIGH THROUGHPUT SCREENING

Figure 4. OptiMSe report – Overview and Gel Plot
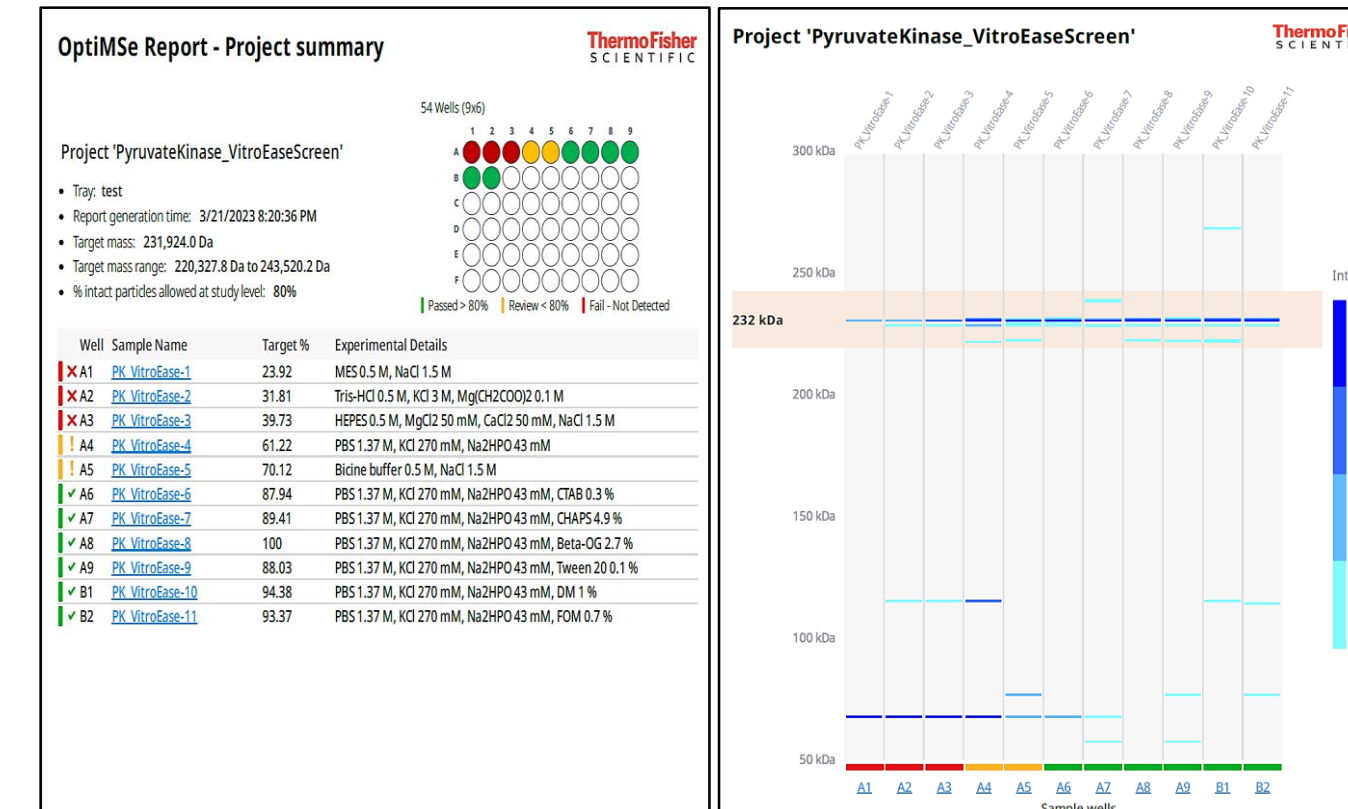
Figures 4 & 5 are taken from a 96 well automated experiment (8 samples x 11 conditions+1 blank). Note: For comparative value, each parent sample is written to its own report such that all conditions of the parent sample can be easily visualized in the gel-plot. Figure 5 shows the Pyruvate Dehydrogenase Kinase complex under two buffer conditions MES+NaCL and PBS+CHAPS from the VitroEase kit. The results clearly shows that the PK complex is more stable in the presence of CHAPS. Importantly there is a strong correlation between the raw data (**fig. 5 top**) and the OptiMSe automated deconvoluted results (**fig. 5 bottom**). Within minutes of acquiring data users can clearly see the impact of buffer composition on PK stability. These results translate into substantial time and resource savings for downstream CryoEM sample prep and microscopy by avoiding unstable sample preparations.

Figure 5. OptiMSe report – Correlation of raw and deconvoluted results
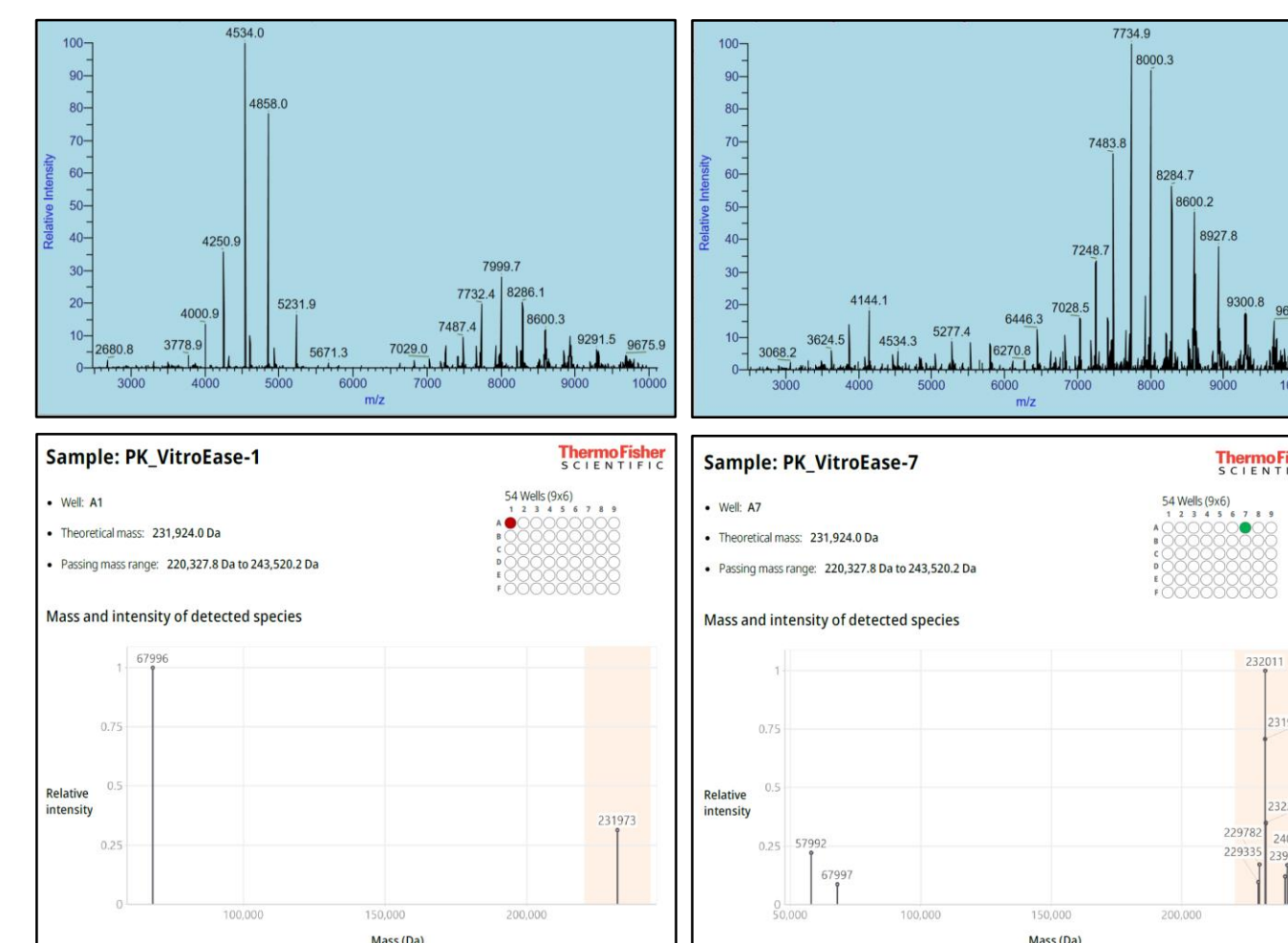
ONLINE MEMBRAME PROTEIN SCREENING

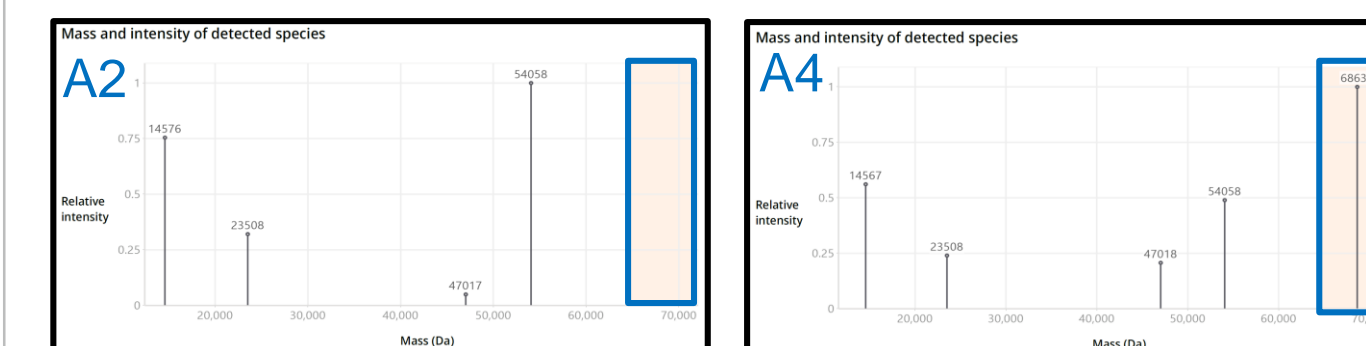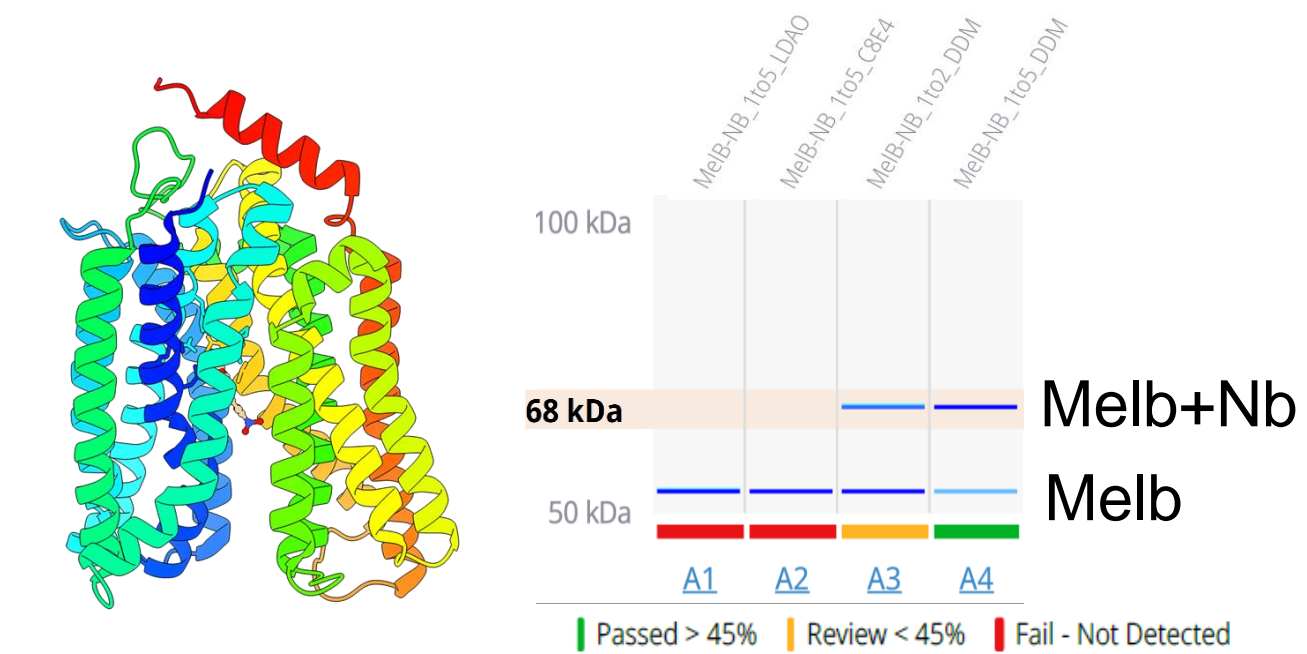### Figure 6. MelB-NanoBody complex buffer screening

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 nanobody to MelB[1].

CONCLUSIONS

The OptiMSe 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 at scale.
- High throughput analysis allows for more experimental space to be covered during sample preparation (including buffer selection).

REFERENCES

1. Lan G et al. A nanobody-trapped novel conformation of a melibiose transporter MelB by cryo-EM single-particle analysis, COMPA, 2022, NYC

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TRADEMARKS/LICENSING

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