Automated native mass spectrometry screening of membrane proteins for structural biology applications

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ABSTRACT

Purpose: Development of a fully automated workflow for screening cryo-EM samples using native mass spectrometry for non-experts.
Methods: Experiments were set up using a custom-build application on the cryo-EM data management platform Athena, which allowed the structural biology user to input basic information, which is used to set up the instrument tuning for the QExactive UHMR mass spectrometer and allowed triggering of unguided, automated data analysis of experimental data.
Results: We demonstrated how after optimization of the desolvation conditions the workflow can be used in an automated fashion.

INTRODUCTION

The last five years a sharp increase in membrane protein structures has been solve, driven largely by the adoption of cryo-EM. Cryo-EM is specifically well positioned to solve previously unobtainable integral membrane proteins (IMPs) structures like GPCRs[1]. 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 due to the heterogeneity introduced by the membrane mimetics required for stabilization of the membrane protein. Here we present a fully automated native mass spectrometry-based workflow, designed for non-experts, to screen membrane proteins for stability, homogeneity and composition.

MATERIALS AND METHODS

Samples

- Purified protein and protein complex samples ranging in mass from Bovine Carbonic Anhydrase II (CA, 29 kDa) to GroEL (801 kDa)
- Pierce Intact Protein Standard Mix (P/N A33526)
- Recombinant *Salmonella typhimurium* MelBst and Nb725m[2] were provided by Prof. Lan Guam, Texas Tech University

LC-MS methods

Thermo Scientific™ Vanquish™ Flex UHPLC system

Thermo Scientific™ Q Exactive™ UHMR Orbitrap™

Column: NativePac OBE-1 (P/N 43803-052130) 80 Å, 2.1 mm x 5 cm

Mobile phase: 50-200 mM Ammonium Acetate with/out 2CMC of LDAO or DDM detergents.

Cryo-EM

- $MelB_{St}$ was reconstituted into lipid nanodisc using MSP 1D1E3 before being mixed with a complex of Nb725m/NabFab/eNb at a ratio of 1:1.5.
- Grids were prepared by Vitrobot Mark IV, and cryo-EM single particles were imaged by Titan Krios TEM with a K3 detector at S^2C^2, Stanford, CA.

Data Analysis

1. Sample queues generated with Thermo Scientific™ Athena Software platform.
2. Acquired data with Thermo Scientific™ Xcalibur™ software on the mass spectrometer.
3. Process data with in-house algorithms in real time
4. Results are presented to the user via high level summaries and detailed mass spectrometry-based reports.

RESULTS

Cryo-EM Workflow: Protein-to-Structure

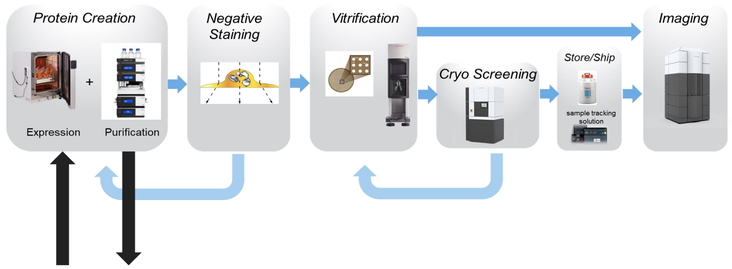
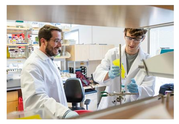
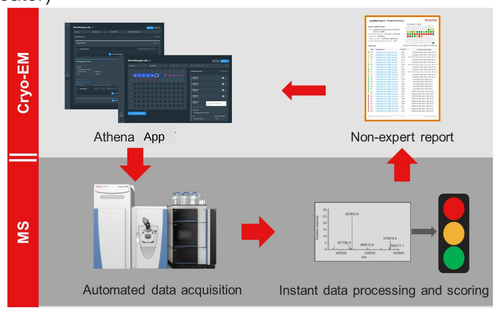


Figure 1. Two challenges/Two solutions
In many cases, protein chemists generating samples for Cryo-EM are new to the field of mass spectrometry and team up with expert users or core facilities to run their samples. We have decoupled the sample introduction process and tailored it to the scientist.

Structural Biologist (Sample Creator)



Mass Spec Operator (Analyst)



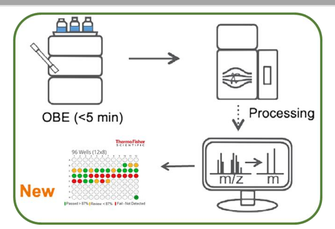
Automated Mass Spectrometry Analysis

. Online Buffer Exchange (OBE)

- OBE separates salt from proteins
- Less than 5 min/sample
- High-throughput screening

Adaptive Analysis

- Fine tuning based on sample input
- Applies optimized sets of analysis conditions
- Applies optimized data analysis algorithms
- Results reported via PDF
- Results in Athena Software for remote users



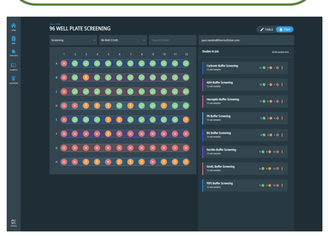
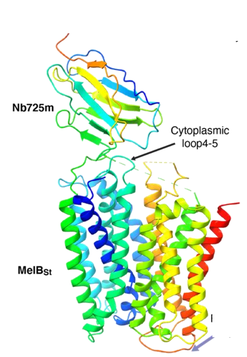


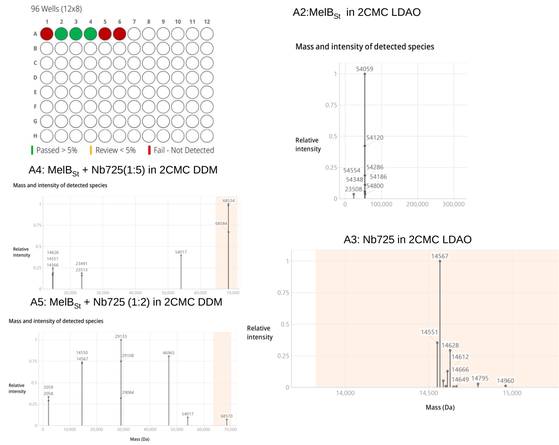
Figure 2. Examples of results in Athena app.

Workflow validation for MelBst-Nb725 complex

Figure 3. OptiMse Analysis of MelBst-Nb725 complex in presence of Na.

$MelB_{St}$, a member of the major facilitator superfamily (MFS), catalyzes a coupled symport of galactoside with Na^+, Li^+, or H^+. Nb725m binds to $MelB_{St}$ at 1.92 µM and stabilized its inward structure[2]. Low binding affinity exhibits additional challenges for automated workflow





CONCLUSIONS

OptiMSe workflow can filter out poor quality samples

Adding OBE-nMS screening to a Cryo-EM pipeline improves the sample-to-structure generation rate.

- High throughput screening removes least promising samples from the queue allowing for higher yield of Cryo-EM structures.
- 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 cover during sample preparation (including buffer section).

REFERENCES

1. Danev, R., Belousoff, M., Liang, YL. *et al.* Routine sub-2.5 Å cryo-EM structure determination of GPCRs. *Nat Commun* **12**, 4333 (2021). https://doi.org/10.1038/s41467-021-24650-3
2. 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