thermoscientific

Establishing a Decision Tree for Native Mass Spectrometry Analysis of Membrane Proteins in Complex Membrane Mimetics

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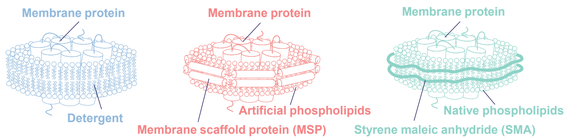
INTRODUCTION

Membrane proteins (MPs) represents 60% clinical drug targets owing to their active involvement in cellular processes. The complexity of membrane mimetics for MPs solubilization pose the challenges for native mass spectrometry (nMS) analyses. Common membrane mimetics including detergent micelles, nanodiscs and styrene maleic-acid lipid particles (SMALPs) are arguably critical for preserving native structure of MPs.

Here, we aim to develop a decision tree for nMS characterization of MPs in different membrane mimetics. Firstly, online buffer exchange-nMS (OBE-nMS) enables quick assessment of MPs in either detergent or nanodiscs. Secondly, either charge detection mass spectrometry or proton transfer charge reduction is compelling to resolve MPs in SMALPs. Decision tree approach using variety of MPs demonstrates its complementarity to other structural biology tools.



MATERIALS AND METHODS

Test Methods

Direct Mass Technology mode employs direct infusion with Thermo Scientific™ Nanospray Flex™ ion source coupled to Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ Mass Spectrometer equipped with an ExD cell (e-MSion, Inc.).

Online buffer exchange was performed by using a Thermo Scientific™ Vanquish™ Flex UHPLC system and Thermo Scientific™ NativePac OBE-1 Column (p/n 43803-052130). Mobile phase is 200 mM ammonium acetate w/ or w/o detergent in it.

Data independent data acquisition - proton transfer charge reduction (DIA-PTCR) was performed on Thermo Scientific™ Orbitrap™ Ascend Tribrid™ Mass Spectrometer.

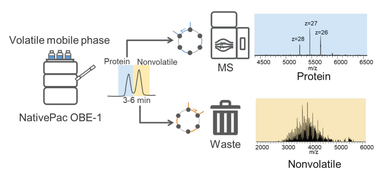


Figure. Online buffer exchange-native MS (OBE-nMS) workflow

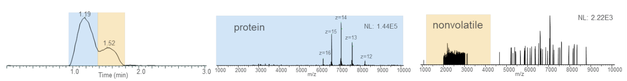
Data Analysis

Data were analyzed using Thermo Scientific™ BioPharma Finder™ 5.1 Software and STORIboard processing software (Proteinaceous).

RESULTS

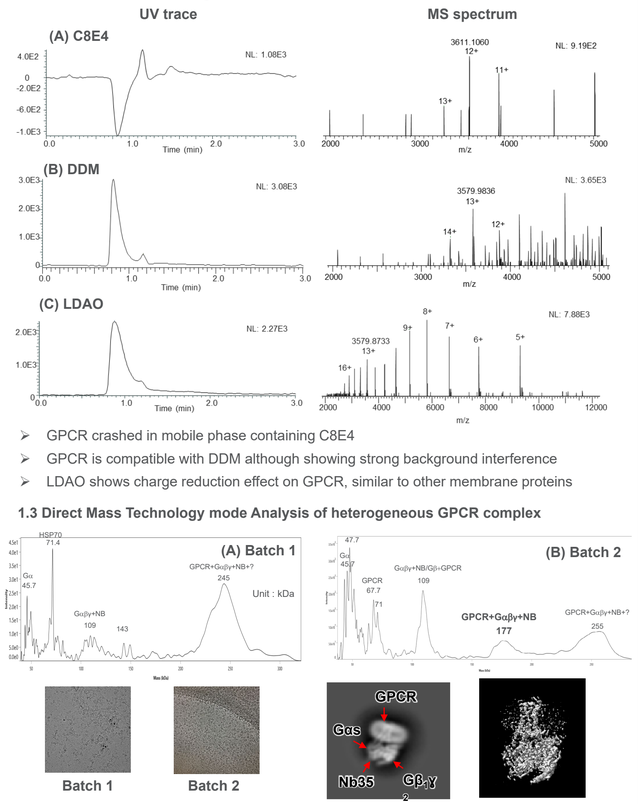
1. Membrane Proteins in Detergent

1.1 OBE-nMS of Membrane Proteins in Detergent



- OBE completes screening of membrane protein within 3-min by separating nonvolatile from membrane protein
- By using ammonium acetate as mobile phase, spectra shows some soluble proteins

1.2 Mobile Phase Screening for OBE-nMS of GPCR



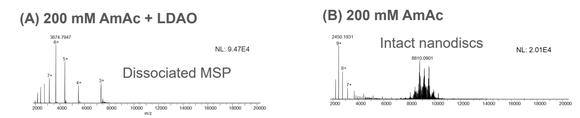
- GPCR crashed in mobile phase containing C8E4
- GPCR is compatible with DDM although showing strong background interference
- LDAO shows charge reduction effect on GPCR, similar to other membrane proteins

1.3 Direct Mass Technology mode Analysis of heterogeneous GPCR complex

- Batch 2 shows a new species ~177 kDa correlated to target MW of complex
- Direct Mass Technology mode analysis aligns with Cryo-EM analysis

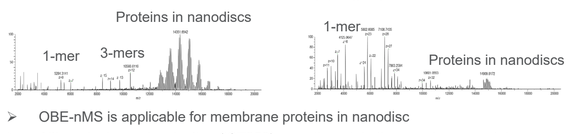
2. Membrane Proteins in Nanodiscs

2.1 Mobile Phase Selection for OBE-nMS of Empty Nanodiscs



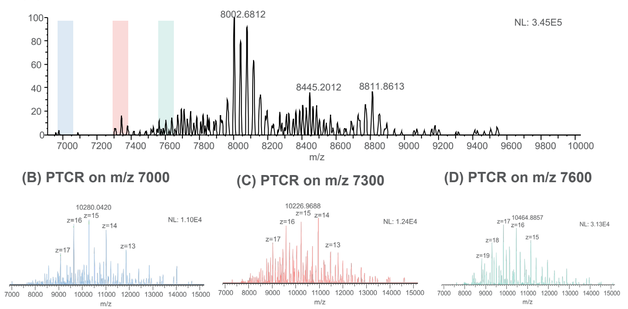
- Mobile phase containing LDAO dissociates nanodisc assembly into membrane scaffold protein monomer
- Nanodiscs remain intact by using 200 mM AmAc as mobile phase plus EASY-Spray 15 μm emitter

2.2 OBE-nMS of Membrane Proteins in Nanodiscs



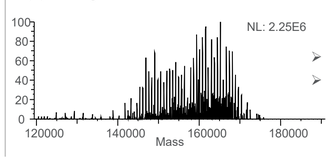
- OBE-nMS is applicable for membrane proteins in nanodisc
- Although strong desolvation (IST250) may dissociate the membrane protein complex

2.3 DIA-PTCR of Membrane Proteins in Nanodiscs



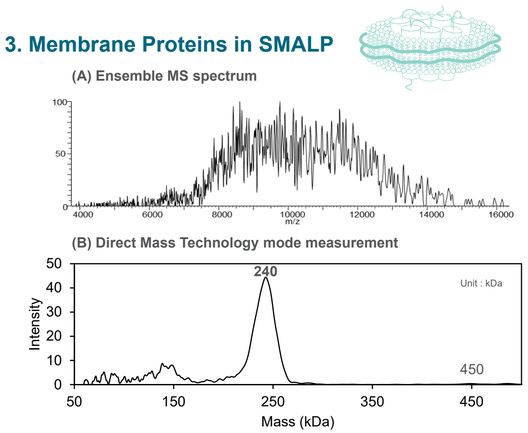
- PTCR resolves the charge states disclosing numerous hidden peaks in the full scan

(E) Sliding window deconvolution of DIA-PTCR spectrum



- Deconvolution gives MW profile ~140-175 kDa
- Space between adjacent deconvoluted peaks 700-750 Da fall into lipid MW range

3. Membrane Proteins in SMALP



- Ensemble MS shows big hump from m/z 4,000-16,000
- Direct Mass shows MW profile ~240 kDa, protein dimer plus SMALP, correlating with SEC-MALS(255+/-9kDa)

CONCLUSIONS

Membrane proteins in detergent

- Use LDAO+200 mM AmAc as mobile phase for initial OBE-nMS rapid screening
- If charge state unresolved, use Direct Mass Technology for target MW>100 kDa or DIA-PTCR for target MW<100 kDa

Membrane proteins in nanodiscs

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Membrane proteins in SMALP

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TRADEMARKS/LICENSING

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