Online Buffer Exchange Enables Automated Membrane Protein Analysis by Native Mass Spectrometry

Weijing Liu[1,†], Hiruni S. Jayasekera[2,†], James D. Sanders[2], Guozhi Zhang[2], Rosa Viner[1,*], Michael T. Marty[2,*]

[1] Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134

[2] Department of Chemistry and Biochemistry and Bio5 Institute, University of Arizona, Tucson, Arizona 85721

[†] These authors contributed equally

Abstract

Membrane proteins represent the majority of clinical drug targets and are actively involved in a range of cellular processes. However, the complexity of membrane mimetics for membrane protein solubilization poses challenges for native mass spectrometry (MS) analyses. The most common approach for native MS analyses of membrane proteins remains offline buffer exchange into native MS-compatible buffers prior to manual sample loading into static nano-ESI emitters. This laborious process requires relatively high sample consumption and optimization for individual proteins. Here, we developed online buffer exchange coupled to native mass spectrometry (OBE-nMS) for analyzing membrane proteins in different membrane mimetics, including detergent micelles and nanodiscs. Detergent screening for OBE-nMS reveals mobile phases containing ammonium acetate with lauryl-dimethylamine oxide is most universal for characterizing both bacterial and mammalian membrane proteins in detergent. Membrane proteins in nanodiscs simply require ammonium acetate as the mobile phase. To preserve the intact nanodiscs, a novel switching electrospray approach was used to capture the high-flow separation on column with a low-flow injection to MS. Rapid OBE-nMS completes each membrane protein measurement within minutes and thus enables higher-throughput assessment of membrane protein integrity prior to its structural elucidation.

Introduction

Membrane proteins play important physiological roles and are major pharmaceutical targets.[1,2] Lipids surrounding membrane proteins can modulate their structure and function in bilayers.[3,4] Prior research has revealed that these membrane protein-lipid interactions are specific and thus control the structural stability, folding and insertion, and the oligomerization of their assemblies.[3,5,6] Despite the challenges associated with characterizing membrane proteins, investigating their structure, stability, and function in bilayer environments is crucial to understand these interactions.[7,8]

Recently, native mass spectrometry (MS), which uses nondenaturing electrospray ionization conditions to preserve noncovalent interactions, has emerged as a valuable approach to study membrane protein interactions and oligomerization.[4,9–12] The buffer composition, including salts and detergents, can influence the structure, function, and interactions of proteins. Buffer components can also play an important role in achieving quality native MS data.[13]

Due to the incompatibility of native MS with nonvolatile buffer components, conventional workflows usually require a buffer exchange/desalting step to transfer the analyte into a native MS-compatible buffer, usually ammonium acetate (AmAc), and to remove any small molecule contaminants. Traditionally, this step is performed offline using size exclusion chromatography, spin columns, molecular weight cutoff filters, or dialysis, which all add significant time and effort. Membrane proteins also need a detergent or membrane memetic to keep the protein solubilized. After offline sample preparation, the sample is typically manually loaded into a single-use static nanospray needle for ionization. Recently, online buffer exchange-native mass spectrometry[14–16] (OBE-nMS) has enabled automated desalting and online injection into the mass spectrometer of soluble proteins. Variations of OBE-nMS have been adapted to high-throughput screening applications and automated purification and confirmation of recombinantly expressed proteins.[17,18]

Here, we develop OBE-nMS methods for membrane proteins. First, we use AmAc buffer containing detergents as the mobile phase for the analysis of membrane proteins in detergents. Second, we use detergent-free mobile phase for membrane proteins embedded in nanodiscs. Finally, to improve ionization gentleness, we interfaced higher flow (100 µl/min) OBE separation with a lower flow (5-15 µl/min) electrospray ionization (ESI) using the Thermo Fisher EASY-Spray™ source. Together, these methods advance automated analysis of membrane proteins by native MS prior to structural analysis by cryo-EM.

Methods

Chemicals

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-racglycerol) (POPG), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) lipids were purchased from Avanti Polar Lipids. AmAc, Amberlite XAD-2, and sodium cholate were purchased from Sigma-Aldrich. Tetraethylene glycol monooctyl ether (C8E4), *n*-dodecyl-β-D-maltoside (DDM), and ammonium acetate were purchased from Sigma-Aldrich. Lauryl-dimethylamine oxide (LDAO) was purchased from Anatrace.

Protein Expression and Purification

Membrane scaffold protein, MSP1E3D1, was expressed and purified in *E. coli* as previously described.[19–22] AqpZ-TEV-GFP-HIS and HIS-MBP-TEV-AmtB membrane proteins were expressed in *E. coli* and purified as previously described using immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC).[19,20,23] Both proteins were TEV-cleaved with TEV protease upon incubation at 4 ˚C overnight and buffer exchanged into 0.2 M AmAc buffer with 0.5% C8E4 using SEC with a Superose 6 10/300 Increase GL (Cytiva). Initial preparation with native MS-compatible conditions allowed us to perform offline controls. Peak fractions were concentrated to 1–10 µM with 50k molecular weight cutoff (MWCO) filter. Samples were flash frozen at -80 ˚C until the analysis. The adenosine 2A receptor (A2AR) was prepared as previously described[24] and was generously provided by Dr. Idlir Liko and Dr. Hsin-Yung Yen from OMass Technologies.

Nanodisc Assembly and Purification

Empty nanodiscs were assembled and purified as previously described.[19,21] Briefly, DPPC was dissolved in chloroform, quantified using phosphate analysis, and dried under N_2 gas and vacuum

overnight.[25] Dried lipids were resolubilized in 0.1 M sodium cholate and MSP1D1(-) were mixed together and were incubated at 4 °C for 1 hour. Then, Amberlite XAD-2 beads were added to the same mixture and incubated overnight in a tube rotator at 37 °C. Upon nanodisc self-assembly, nanodiscs were purified using Superose 6 10/300 Increase GL (Cytiva) with 0.2 M AmAc.

AmtB nanodiscs were assembled using lipids and MSP1E3D1(−), and purified as previously described.[1,21,23] Briefly, POPC and POPG lipids were dissolved in chloroform and quantified using phosphate analysis.[25] Quantified lipids were mixed together to make mixed lipids at 1:1 ratio and were dried with N_2 and under vacuum overnight. Dried lipids were resuspended in 0.1 M sodium cholate. POPC/POPG (50/50) lipids in cholate, and MSP were incubated for 5 minutes on ice, and AmtB was added to the mixture and incubated at 4 °C for 1 hour. Then, Amberlite XAD-2 beads were added to the same mixture and incubated overnight in a tube rotator at 4 °C. Upon nanodisc self-assembly, IMAC was performed to remove empty nanodiscs without the membrane protein, and the membrane protein nanodisc fractions were purified using SEC with a Superose 6 10/300 Increase GL (Cytiva). Peak fractions from the SEC were added with TEV protease and was incubated overnight at 4 °C to cleave the HIS-MBP tag of AmtB. Upon TEV cleavage, the nanodiscs were purified using another round of IMAC and SEC. The final peak fractions were pooled together and was concentrated to a final concentration of 1–5 µM. Samples were flash frozen at -80 °C until the analysis.

Online Buffer Exchange

Online buffer exchange coupled to native MS was performed using a Thermo Scientific™ Vanquish™ Flex UHPLC system with duo pumps coupled to Thermo Scientific™ Q Exactive™ UHMR. The column for online buffer exchange was a Thermo Scientific™ NativePac OBE-1 SEC column for Online sample preparation. The mobile phases were 50–200 mM AmAc containing different detergents for analyzing membrane proteins in detergent micelles. Detergents were used at either 0.5% C8E4 or 0.05% LDAO, which are each roughly twice the critical micelle concentration (CMC). The mobile phase was 200 mM AmAc only (no detergents) for analyzing empty nanodiscs and membrane proteins in nanodiscs. Buffer exchanged membrane proteins eluted first and were directed to the MS. Subsequent non-volatile salts eluted later and were diverted to waste by a six-port valve.

Two different ionization configurations/sources were tested: HESI and EASY-Spray (**Figure 1A** and **1B**, respectively). We used the HESI source with a flow rate of 100 µL/min both on-column and to-MS for detergent-based experiments. We used the EASY-Spray™ source with a flow rate of 100 µL/min on-column but 5–15 µL/min to-MS for nanodisc experiments. Here, a 5 µL sample loop was connected on a six-port valve for switching between high and low flow. The desired elution peak from the high-flow separation was captured in the sample loop prior to injection on the MS using the lower flow pump, analogous to heart-cutting separations schemes.
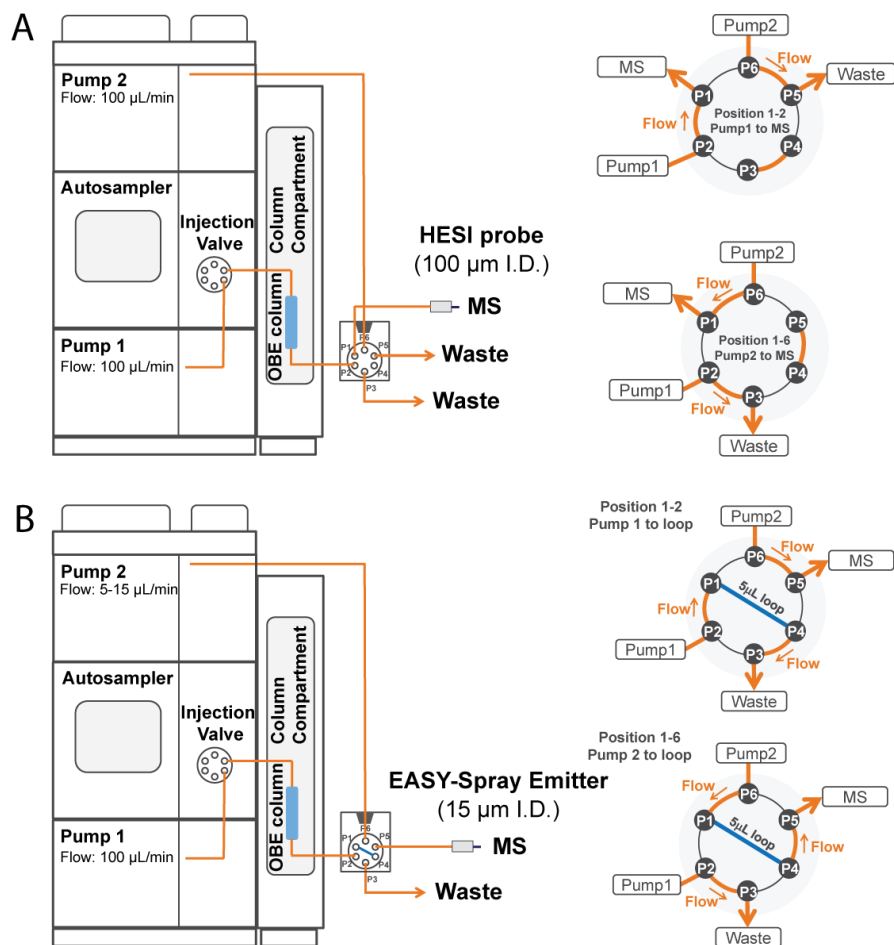
Figure 1. OBE-nMS with (A) HESI and (B) EASY-Spray ion configurations.

Native MS Analysis

Native MS analyses were performed on Thermo Scientific™ Q Exactive™ UHMR. Spray voltage was set at 4.0–4.3 kV for HESI source and 2.0–2.8 kV for EASY-Spray source. The capillary temperature was set at 275 to 300 °C. Ion transfer target m/z and detector optimization were set to "high m/z" and "low m/z," respectively. In-source trapping was set at −50 to –250 V depending on the analytes. Data was acquired at resolution settings of 6,000–12,000 at m/z 400 with transient averaging enabled. The m/z range was adjusted based on the specific sample.

Native MS Data Analysis

Data analysis was performed using UniDec.[26] For membrane proteins in detergents, deconvolution settings included a mass range 5–200 kDa, a charge range of 1–25, and the masses were sampled every 10 Da. The peak FWHM was adjusted between 1–5 Th. The charge smooth width and point smooth width were set to 1, except the charge smooth width of AqpZ was adjusted to -1 as needed. Artifact suppression was also used on some data sets.

For the EASY- and HESI-Spray comparison with empty nanodiscs, the mass range was set to 10–200 kDa, the charge range was 1–18, and the masses were sampled at every 10 Da. Peak FWHM was set to 10.0 Th, and the lipid mass 733.5 Da for DPPC was used as the mass difference. The beta value was set to 15.0. Charge smooth width, point smooth width, and mass point width were all set to 1. For the AmtB nanodisc analysis, deconvolution settings included a mass range of 2–300 kDa, charge range of 7–20, and peak FWHM of 10.0. Charge smooth width was set at -1, and both point and mass smoothing widths were set at 1. Mass differences were set to 755 Da and masses were sampled every 5 Da.

Results and Discussion

Here, our goal was to develop OBE-nMS for the analysis of membrane proteins and their complexes. A fundamental challenge in all membrane proteins native MS workflows is their inherent insolubility in aqueous buffers. Typically, this challenge is resolved using detergents or membrane mimetics like nanodiscs, which solubilize the hydrophobic membrane proteins and provide them with a native-like environment.[10,27,28] Therefore, we tested the OBE-nMS workflow on membrane proteins in both detergent and nanodiscs.

OBE-nMS of Membrane Proteins in Detergent

Detergents aid in the solubilization and stability of membrane proteins in aqueous buffers, making them compatible with native MS analysis. Volatile salts such as AmAc play another crucial role by providing a physiological ionic strength for the solution and establishing the pH.[29] Previous studies have shown that the type of detergent used and salt concentrations both have significant effects on membrane protein oligomeric states and lipid binding characteristics.[10,23,30–32] Therefore, to develop OBE for membrane protein analysis, we first exchanged the membrane protein, AqpZ, into mobile phases containing different amounts of detergents and salts.

We began by using mobile phases with and without detergent to solubilize membrane proteins for buffer exchange and native MS analysis. As expected, the target protein (AqpZ) is not detected without detergents, and the spectrum contains only low abundance co-purified soluble proteins: TEV protease at 28 kDa and DnaK at 69 kDa (**Figure 2A and 2D**). In contrast, with C8E4 detergent added at twice the CMC to the 100 mM AmAc mobile phase, both the 24 kDa monomer and 98 kDa tetramer of AqpZ gave abundant signal (**Figure 2B and 2E**). Increasing the AmAc concentration to 200 mM while maintaining the detergent concentration at 0.5% yielded a spectrum of almost entirely tetramer (**Figure 2C and 2F**). These data demonstrate the importance of detergents for the successful OBE-nMS of membrane protein complexes in the absence of a stable membrane mimetic. Moreover, they reveal how the ionic strength of the mobile phase affects the oligomeric state of AqpZ observed by native MS.

The nature of the detergent used plays an important role in preserving the native conformations of membrane proteins and improving the quality of mass spectra, as reviewed previously.[30] To investigate these differences, we compared C8E4 with another popular detergent, LDAO. Because saccharide detergents, such as DDM, often introduce high background signals, these two detergents have become commonly used for membrane protein analysis due to their excellent compatibility with native MS.[23,30,31] With OBE, there were also excellent signals for both AqpZ (**Figure 3A and 3D**) and AmtB (**Figure 3B and 3E**) in both detergents. As expected, LDAO had greater charge reduction effects on both membrane proteins compared to C8E4.[31,33] Slightly more dissociated monomer was observed for C8E4 compared to LDAO, suggesting LDAO may better for native MS of these proteins.
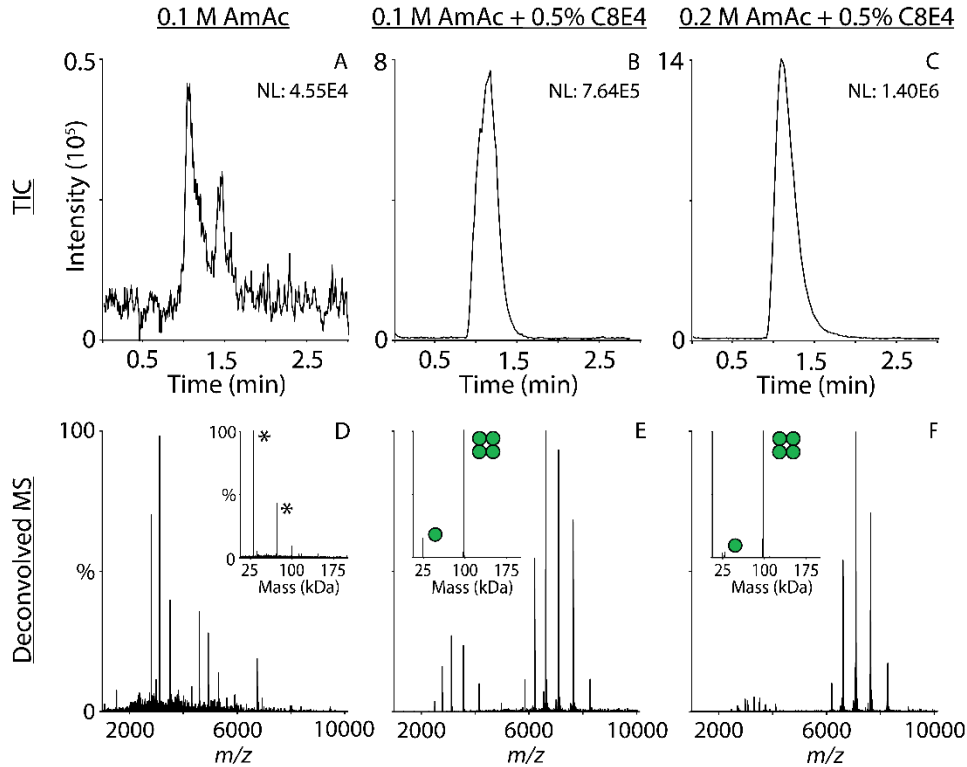
Figure 2. OBE–nMS analysis of AqpZ with and without detergent in the mobile phase including the total ion chromatograms (A–C) and raw mass spectra (D–F) with deconvolved spectra shown as *insets*. (A, D) OBE-nMS using 100 mM AmAc without detergent has only contaminant peaks at 28,050 Da (TEV protease), 68,980 Da (DnaK), and 95,520 Da (unknown). (B, E) OBE-nMS using 100 mM AmAc with 0.5% C8E4 detergent gives mass peaks at 24,710 Da and 98,870 Da for AqpZ monomer and tetramer, respectively. (C, F) OBE-nMS using 200 mM AmAc with 0.5% C8E4 detergent yields predominantly tetramer. Oligomeric states of AqpZ monomer and tetramer are depicted by one and four green circles, respectively, and contaminant proteins are marked with an *.

However, when examining a less stable mammalian membrane protein, the selection of appropriate detergent became more critical. The G-protein coupled receptor (GPCR) A2AR was incompatible with C8E4 in the mobile phase and did not show any clear signal for the target protein, only showing two unknown contaminant mass peaks at 43,320 Da and 50,130 Da. (**Figure 3C**). In contrast, mobile phase containing LDAO showed clear MS signal for monomeric A2AR (**Figure 3F**). Overall, these data demonstrate that OBE-nMS is not only applicable for transporters from bacteria but also GPCRs from mammalian cells. From these data, LDAO was the best detergent to generate high quality membrane protein spectra across the board.
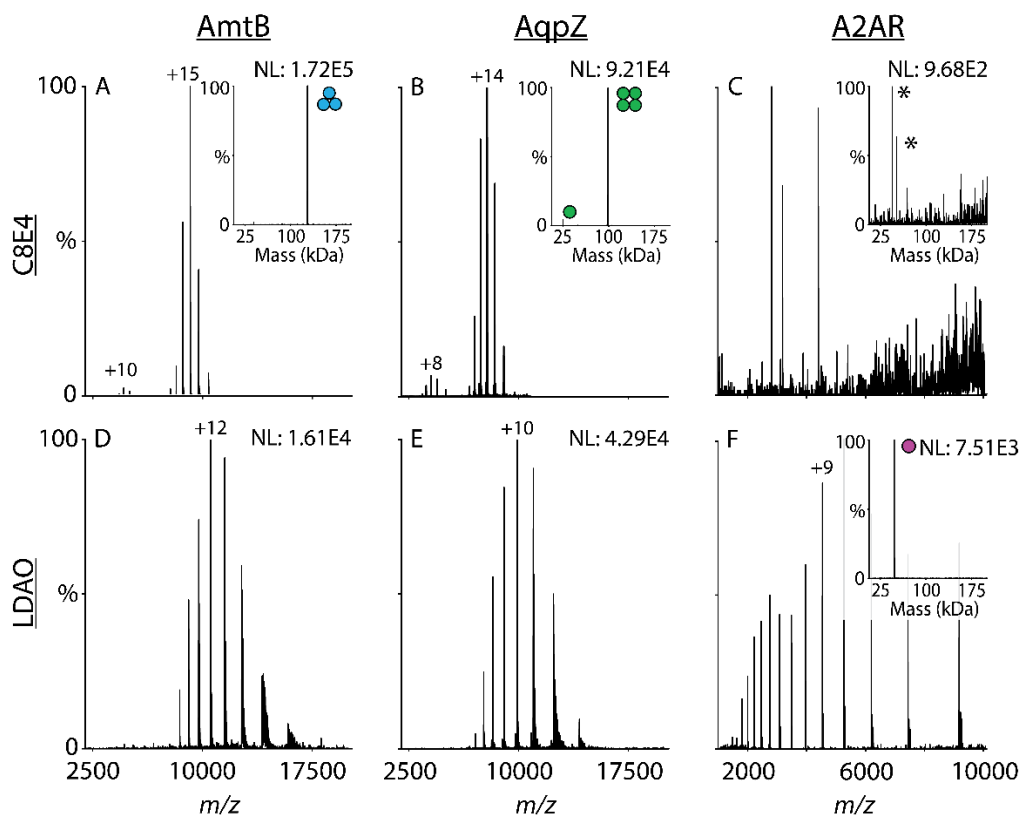
Figure 3. OBE-nMS of AmtB (A, D), AqpZ (B, E), and A2AR (C, F) with 0.5% C8E4 (A–C) and 0.05% LDAO (D–F) detergents. 200 mM AmAc was used with each detergent, except A2AR used 100 mM AmAc for C8E4 detergent. Charge states of major distributions are annotated, and insets show deconvolved spectra in respective detergent conditions. Circles annotate the oligomeric state of the protein complex, and * mark contaminants.

OBE-native MS of Nanodiscs

Lipoprotein nanodiscs represent an alternative to detergents for solubilizing membrane proteins and can create a local environment more like their native membrane environments. Moreover, due to their heterogeneity, narrow size distribution, and stability, nanodiscs are uniquely suited for native MS to study membrane protein-lipid interactions.[10,19,34] Nanodiscs can be prepared either with (filled) or without (empty) a membrane protein incorporated and typically contain varying numbers of lipids.[23] We initially tested empty nanodiscs with LDAO in the mobile phase and observed dissociated monomer of membrane scaffold protein, which demonstrates that OBE can be used to disassemble nanodiscs online (**Figure S1**).

Using AmAc mobile phase without detergents successfully preserved the intact nanodiscs for native MS (**Figure 4**). The mass spectra of empty nanodiscs have multiple peaks representing different numbers of lipids incorporated in the nanodisc. Deconvolution with UniDec shows a distribution of masses centered around 150 kDa, representing nanodiscs containing 135–160 lipids. However, conventional high flow (100 μl/min) HESI ionization (**Figure 1A**) did not yield as strong of a signal for the nanodiscs as seen with conventional offline static nano-ESI.[19]

To overcome this limitation, we tested OBE of empty nanodiscs using a lower flow (5–15 µL/min) EASY-Spray source (**Figure 1**B). Although both methods observed intact nanodiscs, analysis with the HESI source yielded spectra with substantially weaker signal for the intact nanodisc compared to the EASY-Spray spectra. Using the EASY-Spray source, we observed well-resolved nanodiscs (**Figure 4**) that closely resemble previous offline static nano-ESI data.[19] Overall, the EASY-Spray source produced the most native spectra of empty nanodiscs with the benefits of OBE automation.
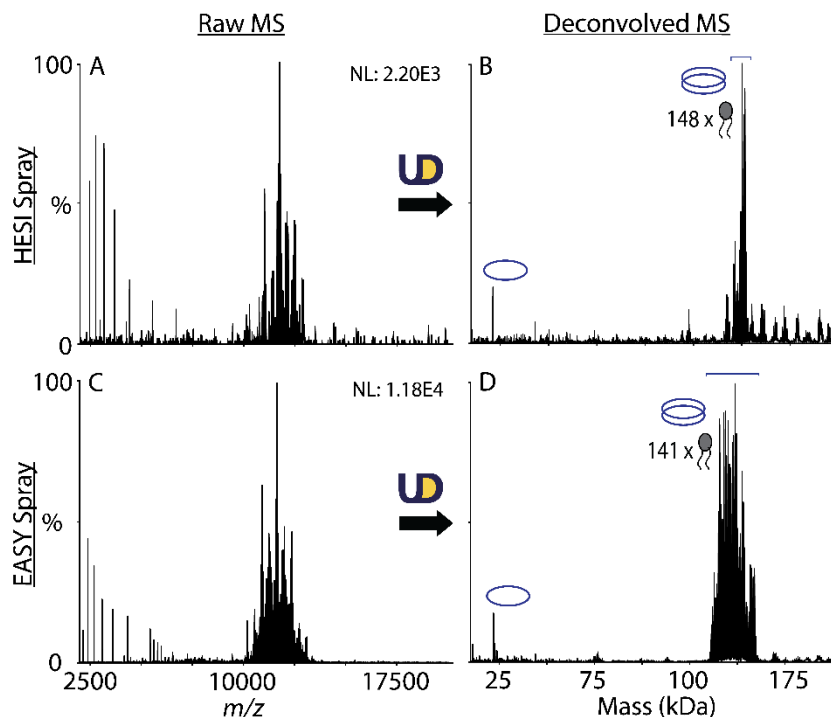

Figure 4. OBE-native MS of empty nanodiscs buffer exchanged into 200 mM AmAc. Raw spectra (A, C) and deconvolved (B, D) spectra collected using either a HESI source with higher flow rates (A, B) or an EASY-Spray source at lower flow rates (C, D). MSP and lipids are depicted in blue and grey graphics, respectively. The average number of lipids per complex is annotated.

Finally, we tested membrane protein nanodiscs containing AmtB. OBE with the EASY-Spray source yielded well-resolved native mass spectra with complex distributions (**Figure 5**). UniDec deconvolution of AmtB data showed predominantly ejected AmtB trimer with around 30–50 bound lipids retained (**Figure 5C**). Minor peaks for AmtB monomers and trimers without bound lipids were also observed. These data match prior studies that observed loss of the MSP belt during native MS, with the major species being the remaining AmtB complex with bound lipids.[19,21] This observation demonstrates the value of high-flow OBE with low-flow MS injections to study membrane proteins in nanodiscs, which removes the need for manual injections with single-use needles.
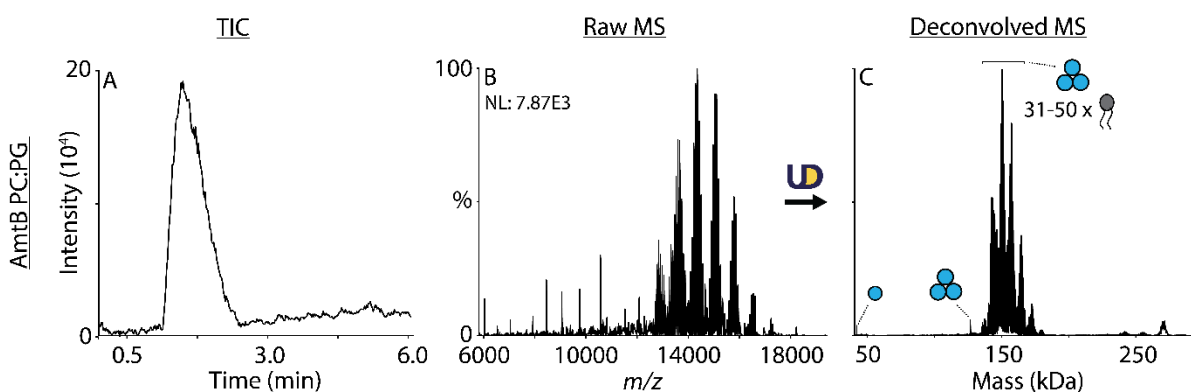
Figure 5. OBE-Native MS of AmtB nanodiscs exchanged into 200 mM AmAc including the (A) TIC, (B) raw spectrum, and (C) deconvolved spectrum. AmtB monomer and trimer are depicted in one and three blue circles, respectively, and the number of lipids is annotated with a grey graphic.

Conclusion

Here, we have shown that OBE-nMS provides an automated solution for analyzing membrane proteins in both detergent micelles and nanodiscs. The selection of proper detergent and buffer conditions for the mobile phase are crucial for the MS analysis of membrane proteins and nanodisc complexes. Specifically, an appropriate concentration of volatile salts in the buffer prevents unintended dissociation of protein complexes, and the choice of detergent determines the survival of membrane proteins after buffer exchange. The use of lower flow rates facilitated by the EASY-Spray source provides more gentle ionization compared to a traditional high-flow HESI source, helping to preserve non-covalent interactions and retain intact nanodisc assemblies. In summary, OBE provides a fast and effective means of exchanging challenging membrane protein samples into a variety of suitable buffers. This approach for online buffer exchange in conjunction with native MS enables higher-throughput analysis of membrane proteins in detergent and nanodiscs and can be a powerful tool for sample screening prior to structural analysis using cryo-EM.

Acknowledgements

Author Information

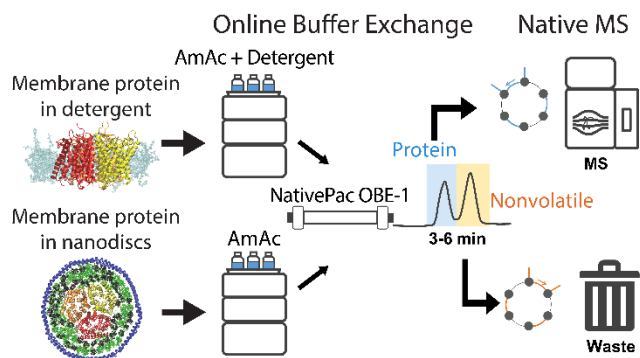* Michael Marty (mtmarty@arizona.edu) and Rosa Viner (rosa.viner@thermofisher.com)

Supporting Information

The Supporting Information includes a supplemental figure.

Conflict of Interest Statement

TOC Graphic

References

(1) Zhang, G.; Keener, J. E.; Marty, M. T. Measuring Remodeling of the Lipid Environment Surrounding Membrane Proteins with Lipid Exchange and Native Mass Spectrometry. *Anal. Chem.* **2020**, *92* (8), 5666–5669. https://doi.org/10.1021/acs.analchem.0c00786.

(2) Arinaminpathy, Y.; Khurana, E.; Engelman, D. M.; Gerstein, M. B. Computational Analysis of Membrane Proteins: The Largest Class of Drug Targets. *Drug Discov. Today* **2009**, *14* (23), 1130–1135. https://doi.org/10.1016/j.drudis.2009.08.006.

(3) Gupta, K.; Li, J.; Liko, I.; Gault, J.; Bechara, C.; Wu, D.; Hopper, J. T. S.; Giles, K.; Benesch, J. L. P.; Robinson, C. V. Identifying Key Membrane Protein Lipid Interactions Using Mass Spectrometry. *Nat. Protoc.* **2018**, *13* (5), 1106–1120. https://doi.org/10.1038/nprot.2018.014.

(4) Bolla, J. R.; Agasid, M. T.; Mehmood, S.; Robinson, C. V. Membrane Protein–Lipid Interactions Probed Using Mass Spectrometry. *Annu. Rev. Biochem.* **2019**, *88* (1), 85–111. https://doi.org/10.1146/annurev-biochem-013118-111508.

(5) Hunte, C. Specific Protein–Lipid Interactions in Membrane Proteins. *Biochem. Soc. Trans.* **2005**, *33* (5), 938–942. https://doi.org/10.1042/BST0330938.

(6) Agasid, M. T.; Robinson, C. V. Probing Membrane Protein–Lipid Interactions. *Curr. Opin. Struct. Biol.* **2021**, *69*, 78–85. https://doi.org/10.1016/j.sbi.2021.03.010.

(7) Cournia, Z.; Allen, T. W.; Andricioaei, I.; Antonny, B.; Baum, D.; Brannigan, G.; Buchete, N.-V.; Deckman, J. T.; Delemotte, L.; del Val, C.; Friedman, R.; Gkeka, P.; Hege, H.-C.; Hénin, J.; Kasimova, M. A.; Kolocouris, A.; Klein, M. L.; Khalid, S.; Lemieux, M. J.; Lindow, N.; Roy, M.; Selent, J.; Tarek, M.; Tofoleanu, F.; Vanni, S.; Urban, S.; Wales, D. J.; Smith, J. C.; Bondar, A.-N. Membrane Protein Structure, Function, and Dynamics: A Perspective from Experiments and Theory. *J. Membr. Biol.* **2015**, *248* (4), 611–640. https://doi.org/10.1007/s00232-015-9802-0.

(8) Levental, I.; Lyman, E. Regulation of Membrane Protein Structure and Function by Their Lipid Nano-Environment. *Nat. Rev. Mol. Cell Biol.* **2023**, *24* (2), 107–122. https://doi.org/10.1038/s41580-022-00524-4.

(9) van Dyck, J. F.; Konijnenberg, A.; Sobott, F. Native Mass Spectrometry for the Characterization of Structure and Interactions of Membrane Proteins. In *Membrane Protein Structure and Function Characterization: Methods and Protocols*; Lacapere, J.-J., Ed.; Methods in Molecular Biology; Springer: New York, NY, 2017; pp 205–232. https://doi.org/10.1007/978-1-4939-7151-0_11.

(10) Keener, J. E.; Zhang, G.; Marty, M. T. Native Mass Spectrometry of Membrane Proteins. *Anal. Chem.* **2021**, *93* (1), 583–597. https://doi.org/10.1021/acs.analchem.0c04342.

(11) Oluwole, A.; Shutin, D.; Bolla, J. R. Mass Spectrometry of Intact Membrane Proteins: Shifting towards a More Native-like Context. *Essays Biochem.* **2023**, *67* (2), 201–213. https://doi.org/10.1042/EBC20220169.

(12) Sahin, C.; Reid, D. J.; Marty, M. T.; Landreh, M. Scratching the Surface: Native Mass Spectrometry of Peripheral Membrane Protein Complexes. *Biochem. Soc. Trans.* **2020**, *48* (2), 547–558. https://doi.org/10.1042/BST20190787.

(13) Susa, A. C.; Xia, Z.; Williams, E. R. Native Mass Spectrometry from Common Buffers with Salts That Mimic the Extracellular Environment. *Angew. Chem. Int. Ed.* **2017**, *56* (27), 7912–7915. https://doi.org/10.1002/anie.201702330.

(14) VanAernum, Z. L.; Busch, F.; Jones, B. J.; Jia, M.; Chen, Z.; Boyken, S. E.; Sahasrabuddhe, A.; Baker, D.; Wysocki, V. H. Rapid Online Buffer Exchange for Screening of Proteins, Protein Complexes and Cell Lysates by Native Mass Spectrometry. *Nat. Protoc.* **2020**, *15* (3), 1132–1157. https://doi.org/10.1038/s41596-019-0281-0.

(15) Cavanagh, J.; Benson, L. M.; Thompson, R.; Naylor, S. In-Line Desalting Mass Spectrometry for the Study of Noncovalent Biological Complexes. *Anal. Chem.* **2003**, *75* (14), 3281–3286. https://doi.org/10.1021/ac030182q.

(16) Waitt, G. M.; Xu, R.; Wisely, G. B.; Williams, J. D. Automated In-Line Gel Filtration for Native State Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2008**, *19* (2), 239–245. https://doi.org/10.1016/j.jasms.2007.05.008.

(17) Deslignière, E.; Ley, M.; Bourguet, M.; Ehkirch, A.; Botzanowski, T.; Erb, S.; Hernandez-Alba, O.; Cianférani, S. Pushing the Limits of Native MS: Online SEC-Native MS for Structural Biology Applications. *Int. J. Mass Spectrom.* **2021**, *461*, 116502. https://doi.org/10.1016/j.ijms.2020.116502.

(18) Hecht, E.; Obiorah, E.; Liu, X.; Morrison, L.; Shion, H.; Lauber, M. Microflow Size Exclusion Chromatography to Preserve Micromolar Affinity Complexes and Achieve Subunit Separations for Native State Mass Spectrometry. *J. Chromatogr. A* **2022**, *1685*, 463638. https://doi.org/10.1016/j.chroma.2022.463638.

(19) Reid, D. J.; Keener, J. E.; Wheeler, A. P.; Zambrano, D. E.; Diesing, J. M.; Reinhardt-Szyba, M.; Makarov, A.; Marty, M. T. Engineering Nanodisc Scaffold Proteins for Native Mass Spectrometry. *Anal. Chem.* **2017**, *89* (21), 11189–11192. https://doi.org/10.1021/acs.analchem.7b03569.

(20) Keener, J. E.; Zambrano, D. E.; Zhang, G.; Zak, C. K.; Reid, D. J.; Deodhar, B. S.; Pemberton, J. E.; Prell, J. S.; Marty, M. T. Chemical Additives Enable Native Mass Spectrometry Measurement of Membrane Protein Oligomeric State within Intact Nanodiscs. *J. Am. Chem. Soc.* **2019**, *141* (2), 1054–1061. https://doi.org/10.1021/jacs.8b11529.

(21) Marty, M. T.; Hoi, K. K.; Gault, J.; Robinson, C. V. Probing the Lipid Annular Belt by Gas-Phase Dissociation of Membrane Proteins in Nanodiscs. *Angew. Chem. Int. Ed.* **2016**, *55* (2), 550–554. https://doi.org/10.1002/anie.201508289.

(22) Denisov, I. G.; Grinkova, Y. V.; Lazarides, A. A.; Sligar, S. G. Directed Self-Assembly of Monodisperse Phospholipid Bilayer Nanodiscs with Controlled Size. *J. Am. Chem. Soc.* **2004**, *126* (11), 3477–3487. https://doi.org/10.1021/ja0393574.

(23) Keener, J. E.; Jayasekera, H. S.; Marty, M. T. Investigating the Lipid Selectivity of Membrane Proteins in Heterogeneous Nanodiscs. *Anal. Chem.* **2022**, *94* (23), 8497–8505. https://doi.org/10.1021/acs.analchem.2c01488.

(24)   García-Nafría, J.; Lee, Y.; Bai, X.; Carpenter, B.; Tate, C. G. Cryo-EM Structure of the Adenosine A2A Receptor Coupled to an Engineered Heterotrimeric G Protein. *eLife* **2018**, *7*, e35946. https://doi.org/10.7554/eLife.35946.

(25)   Ritchie, T. K.; Grinkova, Y. V.; Bayburt, T. H.; Denisov, I. G.; Zolnerciks, J. K.; Atkins, W. M.; Sligar, S. G. Chapter Eleven - Reconstitution of Membrane Proteins in Phospholipid Bilayer Nanodiscs. In *Methods in Enzymology*; Düzgünes, N., Ed.; Liposomes, Part F; Academic Press, 2009; Vol. 464, pp 211–231. https://doi.org/10.1016/S0076-6879(09)64011-8.

(26)   Marty, M. T.; Baldwin, A. J.; Marklund, E. G.; Hochberg, G. K. A.; Benesch, J. L. P.; Robinson, C. V. Bayesian Deconvolution of Mass and Ion Mobility Spectra: From Binary Interactions to Polydisperse Ensembles. *Anal. Chem.* **2015**, *87* (8), 4370–4376. https://doi.org/10.1021/acs.analchem.5b00140.

(27)   Gault, J.; Donlan, J. A. C.; Liko, I.; Hopper, J. T. S.; Gupta, K.; Housden, N. G.; Struwe, W. B.; Marty, M. T.; Mize, T.; Bechara, C.; Zhu, Y.; Wu, B.; Kleanthous, C.; Belov, M.; Damoc, E.; Makarov, A.; Robinson, C. V. High-Resolution Mass Spectrometry of Small Molecules Bound to Membrane Proteins. *Nat. Methods* **2016**, *13* (4), 333–336. https://doi.org/10.1038/nmeth.3771.

(28)   Marty, M. T.; Hoi, K. K.; Robinson, C. V. Interfacing Membrane Mimetics with Mass Spectrometry. *Acc. Chem. Res.* **2016**, *49* (11), 2459–2467. https://doi.org/10.1021/acs.accounts.6b00379.

(29)   Konermann, L. Addressing a Common Misconception: Ammonium Acetate as Neutral PH "Buffer" for Native Electrospray Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2017**, *28* (9), 1827–1835. https://doi.org/10.1007/s13361-017-1739-3.

(30)   Reading, E.; Liko, I.; Allison, T. M.; Benesch, J. L. P.; Laganowsky, A.; Robinson, C. V. The Role of the Detergent Micelle in Preserving the Structure of Membrane Proteins in the Gas Phase. *Angew. Chem. Int. Ed.* **2015**, *54* (15), 4577–4581. https://doi.org/10.1002/anie.201411622.

(31)   Behnke, J.-S.; Urner, L. H. Emergence of Mass Spectrometry Detergents for Membrane Proteomics. *Anal. Bioanal. Chem.* **2023**. https://doi.org/10.1007/s00216-023-04584-z.

(32)   Townsend, J. A.; Sanders, H. M.; Rolland, A. D.; Park, C. K.; Horton, N. C.; Prell, J. S.; Wang, J.; Marty, M. T. Influenza AM2 Channel Oligomerization Is Sensitive to Its Chemical Environment. *Anal. Chem.* **2021**, *93* (48), 16273–16281. https://doi.org/10.1021/acs.analchem.1c04660.

(33)   Kundlacz, T.; Bender, J.; Schmidt, C. Effects of Non-Ionic and Zwitterionic Detergents on Soluble Proteins during Native Mass Spectrometry Experiments. *Int. J. Mass Spectrom.* **2021**, *468*, 116652. https://doi.org/10.1016/j.ijms.2021.116652.

(34)   Marius, P.; de Planque, M. R. R.; Williamson, P. T. F. Probing the Interaction of Lipids with the Non-Annular Binding Sites of the Potassium Channel KcsA by Magic-Angle Spinning NMR. *Biochim. Biophys. Acta* **2012**, *1818* (1), 90–96. https://doi.org/10.1016/j.bbamem.2011.09.017.