Development of Mass Spectrometry Grade Membrane Protein Standard

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Abstract

As excellent targets for drug therapies, membrane proteins (MPs) are heavily researched in the structural biology field. Mass spectrometry (MS) has been instrumental in further studying and analyzing protein structures of interest. However, determining system suitability and identifying optimal instrument parameters prior to sample testing poses a challenge in the field. Recently, we have developed the Thermo Scientific™ Pierce™ Aquaporin Z (AqpZ) Membrane Protein Standard to validate and tune MS instruments before experimental data acquisition. Here, we describe the development of this standard across various structural biology MS methods to highlight its capabilities at assessing and verifying numerous parameters of the liquid chromatography (LC) and MS systems.

Introduction

MS is valuable in characterizing protein structure and interactions. MPs require membrane mimetics to maintain native structure, making their extraction and purification difficult, time consuming, and low-yield. Without determining instrument suitability and method development beforehand, MS analysis can lead to substantial loss of samples and irreproducible results. The Pierce™ Aquaporin Z Membrane Protein Standard provides a reliable MS standard to evaluate, troubleshoot, and tune the instrument prior to sample analysis. Our optimized extraction and purification of AqpZ generates a clean standard compatible with MS analysis. This standard generates consistent data across MS instruments and techniques, helping provide insight and feedback on system suitability, enabling easier troubleshooting and optimized data acquisition parameters for MP analysis.

Materials and methods

Protein expression & purification

AqpZ was expressed in *E. coli* and extracted via ultracentrifugation and solubilization at 4˚ C overnight. Immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC) were utilized to purify AqpZ, and buffer exchange into 0.05% N,N-dimethyl-n-dodecylamine N-oxide (LDAO) and 200mM ammonium acetate buffer prior to freezing and storage at -80˚ C before MS analysis described below. Purity was determined by SEC and native MS (nMS). SEC data was obtained using the Superdex™ 200 Increase 1/300 GL and analyzed with the Unicorn™ software.

Sample Analysis

nMS Analysis via Direct Infusion

nMS direct infusion data was generated on the Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ mass spectrometer. Spray voltage was set to 1.4 kV for nanoESI source and a capillary temperature of 300˚ C. Ion detector optimization was set to "low *m/z*". In-source trapping (iST) was varied from -10 to -140V for each sample. The Thermo Fisher BCDecon software was used to analyze the data and calculate the assembly ratio of AqpZ in its native state (tetramer) to the overall sample.

nMS Analysis via online buffer exchange (OBE)

OBE coupled to nMS was performed using a Thermo Scientific™ Vanquish™ Flex UHPLC system with dual pumps coupled to a Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ mass spectrometer[1]. The column for OBE was a Thermo Scientific™ NativePac OBE-1 SEC column for online sample preparation. Analysis of the LC-nMS data was performed using the Thermo Scientific™ OptiMSe™ 1.0 software.

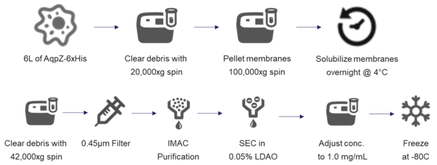
HDX-MS Analysis

AqpZ folding in different detergents was studied by bottom-up HDX-MS experiments using a fully automated HDX workflow station with Vanquish™ Binary pump N and a Thermo Scientific™ Orbitrap Ascend™ Structural Biology Edition mass spectrometer. The data were acquired using a NEPII/Pepsin protease column, 2M Guanidine HCl, 1.5% C8E4 quench, and 2-minute digestion at 200µL/min, and analysed in Thermo Scientific™ BioPharma Finder™, HDExaminer (Sierra Analytics).

Results

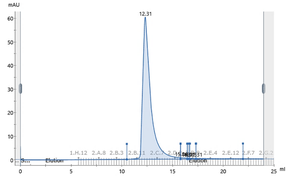
Generating a MS-grade Standard

Figure 1. Optimized purification schematic for AqpZ



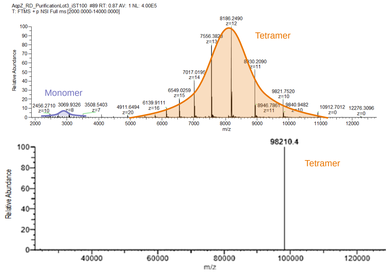
The hydrophobicity of MPs pose a challenge for buffer exchange into a MS-compatible buffer as MPs become unstable, leading to aggregation and loss of protein. Our optimized workflow (Figure 1) produces AqpZ in a ready-to-use format, without compromising sample purity (Figure 2) or native protein structure (Figure 3).

Figure 2. SEC analysis of optimized AqpZ purification reports 97.5% purity.



UV280 SEC chromatogram of 500µL optimized AqpZ preparation final sample run. The single peak demonstrates that the standard sample is uniform and free of aggregates. The retention volume (12.31 mL) indicates the AqpZ is present in its native tetrameric state.

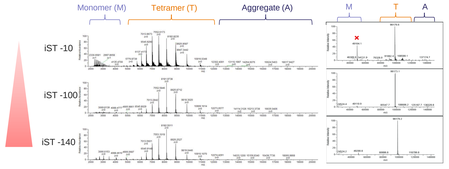
Figure 3. nMS analysis of optimized AqpZ has relative abundance of >98% and confirms native tetrameric structure at 98.2 kDa.



Raw MS spectrum of AqpZ standard (Top) and its deconvolution (Bottom) using static nanospray acquisition of diluted AqpZ on Q-Exactive UHMR.

Parameter Tuning and System Suitability

Figure 4. Direct infusion nMS analysis requires in source trapping (iST).



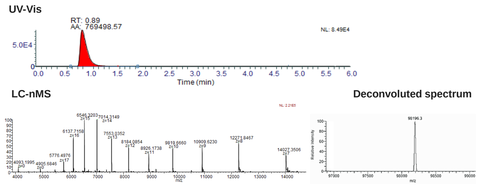
nMS analysis requires careful instrument tuning to resolve envelope(s) and eject MPs from detergent micelles. Testing AqpZ with an iST voltage that is low (iST -10) results in excess noise due to detergent, making deconvolution and resolution of peaks difficult. Higher iST voltage (iST -140) create an artificial increase in AqpZ monomer caused by harsher instrument conditions. By carefully adjusting the iST, background signal is reduced, providing high resolution readings, without impacting protein native state.

Figure 5. Multiple manufacturing lots of AqpZ generate reproducible data via direct infusion, reporting an average fractional abundance of 98.1% tetramer with <5% CV.

Each manufacturing lot of AqpZ standard was tested by direct infusion method and nMS analysis. Fractional abundance (%) was calculated using the BCDecon software. Each manufacturing lot passed the product release specifications for assembly ratio and percent tetramer.

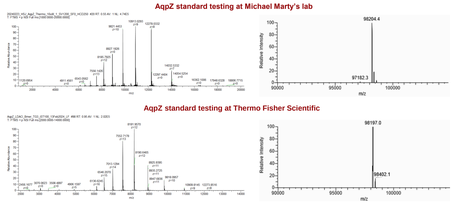
Standard Reproducibility Across Systems

Figure 6. LC-nMS using the Native Pac-1 OBE column provides high throughput method for analysis of MPs.



AqpZ standard was analyzed by OBE-EZ workflow with LDAO in ammonium acetate as a mobile phase. UV-VIS analysis showed a single peak for AqpZ standard. nMS analysis resulted in multiple charge states envelope for AqpZ standard. Deconvoluted spectra calculated using BCDecon software showed tetramer form of AqpZ protein at ~98kDa.

Figure 7. Cross-lab testing of AqpZ standard using the direct infusion method generates reproducible data.



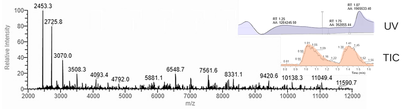
Direct infusion and nMS analysis of a single lot of AqpZ standard was performed at two different sites. Both UHMRs used for the AqpZ testing showed reproducible results exhibiting >98% tetramer form of AqpZ.

Table 1. System Suitability – Failure to meet the below criteria during nMS analysis of the standard is indicative of an unsuitable system.

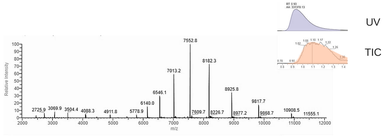
Component	Specification	Criteria
LC	UV Intensity	>2.0e04
LC	Column Pressure	<80 bar ±3
LC	Peak Width (>10% S)	<0.8 min
LC	Peak shape (MS + UV)	1 peak
MS	Tetramer amount	>85%
MS	MS Intensity	>1.0e06

Figure 8. OBE-LC-nMS analysis of the standard sample at iST -100 before and after troubleshooting and maintenance occurred.

(A) Sample testing indicates problems with the LC (more than 1 peak) and MS (tetramer content <85%).

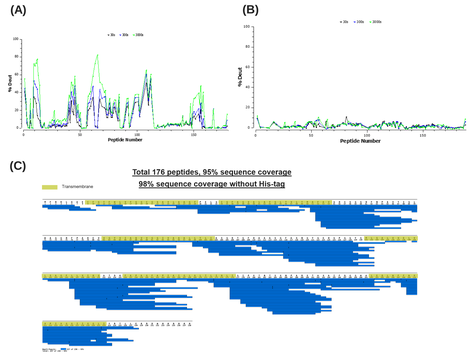


(B) Sample testing confirms system suitability after maintenance and cleaning of the UHMR occurred.



AqpZ standard was used to evaluate LC-nMS system performance before and after preventive maintenance. (A) LC-UV analysis showed more than 1 peak and LC-nMS analysis resulted in higher monomer intensity. (B) LC-UV showed a single peak and LC-nMS analysis resulted in higher intensity of tetramer form of AqpZ.

Figure 9. HDX-MS Analysis of AqpZ in C8E4 (A) and LDAO (B); sequence coverage (C).



Protein identity of AqpZ was confirmed by peptide mapping, and its structure and folding in different detergents was confirmed by HDX-MS experiments. HDX-MS results for AqpZ in C8E4 are consistent with the crystal structure (PDB2ABM), and in LDAO consistent with native MS (Figure 7) as globally more compact structure.

Conclusions

- AqpZ as a mass spectrometry grade membrane protein standard provides robust and reliable results across various MS instruments and test methods.
- The optimized purification workflow consistently generates a clean, ready to use AqpZ sample in its native structure.
- As a standard, AqpZ helps discern system suitability and provides clues to address problems, preventing the loss of precious samples and assists in MPs MS method development for nMS and HDX-MS.

References

1. Liu W, Jayasekera HS, Sanders JD, Zhang G, Viner R, Marty MT. Online Buffer Exchange Enables Automated Membrane Protein Analysis by Native Mass Spectrometry. Anal Chem. 2023 Nov 28;95(47):17212-17219.

Acknowledgements

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