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應用手冊

# Native Mass Spectrometry of Membrane Protein-Ligand Complexes Using the SELECT SERIES™ Cyclic™ IMS

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## Abstract

Membrane proteins are an analytically challenging class of protein that make up 60% of all drug targets.[1] As such, advanced and flexible analytical solutions are required to successfully study them in detail. Native Mass Spectrometry (MS) is gaining traction as a tool to investigate many aspects of membrane protein structure and function, with particular interest in the pharmaceutical industry. Here we highlight the benefits of the SELECT SERIES™ Cyclic™ IMS Mass Spectrometer for performing native MS analysis of membrane proteins. We demonstrate its collisional activation capabilities, required for liberating the proteins from solubilizing detergent micelles, and show how cyclic ion mobility can be used to interrogate the three-dimensional structure of the proteins through measurement of the collision cross section (CCS). Furthermore, we show how the instrument can be used for drug-like screening applications, by detecting small molecule binding to the multidrug and toxin extrusion protein from *pyrococcus furiosus* (*pf*MATE).

## Benefits

- Increase confidence with high quality native mass spectra of membrane proteins using the SELECT SERIES Cyclic IMS Mass Spectrometer
- Track key structural changes in proteins in response to binding or stress using accurate CCS values from cyclic ion mobility
- Enhance drug discovery workflows by identifying small molecule binders in membrane protein-ligand screening studies

## Introduction

Native mass spectrometry, in which biomolecules are electrosprayed from solutions at near physiological pH, is an established technique to investigate protein molecular weight, protein complex stoichiometry, ligand binding, and when enabled with ion mobility spectrometry, higher order structure. However, much of the work ongoing in this field focuses on soluble proteins, for which it is relatively facile to obtain clear high-resolution data. Membrane proteins are more challenging as they must be solubilized in detergents or other agents, which can confound analysis.

Membrane proteins constitute approximately 30% of the human proteome and 60% of all drug targets making them key systems for study in the pharmaceutical and biomedical research areas.[1] For successful analysis by native MS, membrane proteins are typically solubilized in ammonium acetate solution supplemented with a chosen detergent above the critical micelle concentration (CMC). At concentrations above the CMC the detergents form micelles in solution which stabilize the hydrophobic regions of the membrane the protein. Analysis requires the protein-micelle complex to be ionized and transferred into the mass spectrometer. However, the complex is heterogeneous and does not produce informative mass spectra. As such, the solubilizing detergent molecules must be removed within the instrument by high energy collisions, either in the source region or within a collision cell. If successful, the removal of the micelle will yield well resolved protein signals in the mass spectrum.

In this application brief we demonstrate the unique attributes of the SELECT SERIES Cyclic IMS Mass Spectrometer for native MS of membrane proteins. We show how the declustering and activation capabilities of the instrument enable effective micelle removal and yield clear, high-resolution spectra for the bacterial outer membrane protein F (OmpF). We show that ion mobility capability enables the monitoring of protein unfolding,

facilitating optimization of voltages to preserve folded structure and in turn obtain collision cross section (CCS) values of the native-like folded forms of the protein. Furthermore, for the multidrug and toxin extrusion protein from *pyrococcus furiosus* (*pf*MATE) we show how the SELECT SERIES Cyclic IMS Mass Spectrometer can be used for membrane protein-ligand binding studies even from solutions containing over 100 drug-like small molecules.

## Results and Discussion

Firstly, we acquired native mass spectra for a solution of the OmpF protein (4 µM) in a solution of 200 mM ammonium acetate. In this case the solubilizing detergent was 1% β-octyl glucoside (w/v), considered difficult to remove in native MS analyses.[2,3] Figure 1A shows the mass spectrum obtained with typical low energy settings. Under these conditions the instrument is not optimized to decluster OmpF and remove the bound detergent molecules. The spectrum exhibits signals in the low *m/z* region consistent with clusters of the β-OG detergent and in the high *m/z* region (above 6,000 *m/z*) consistent with detergent:protein complexes.

To obtain well-defined signals for OmpF we applied declustering voltage in the source (150 V cone voltage) and in the trap collision cell (200 V). The resulting spectrum (Figure 1B) exhibits a charge state distribution between approximately 4,400 and 5,600 *m/z*. The measured mass is 111.2 kDa, with the distribution centered on the $[M+23H]^{23+}$ species. The well-resolved signals indicate that the protein has been effectively liberated from the detergent micelle.

At the high activation voltages required for detergent removal the folded structure of the protein is likely lost due to collision-induced unfolding processes, meaning it is not possible to determine native-like CCS values. However, ion mobility capability enables unfolding to be monitored meaning that softer conditions can be optimized, unfolding can be minimized (Figure 1B, inset), and CCS values can be measured. Using the IMScal calibration tool (imscal.on-demand.waters.com) the $^{TW}CCS_{N2}$ value for the 21+ charge state of OmpF was determined to be 6,380 ± 52 $Å^2$, consistent with a folded protein of this molecular weight.[4]

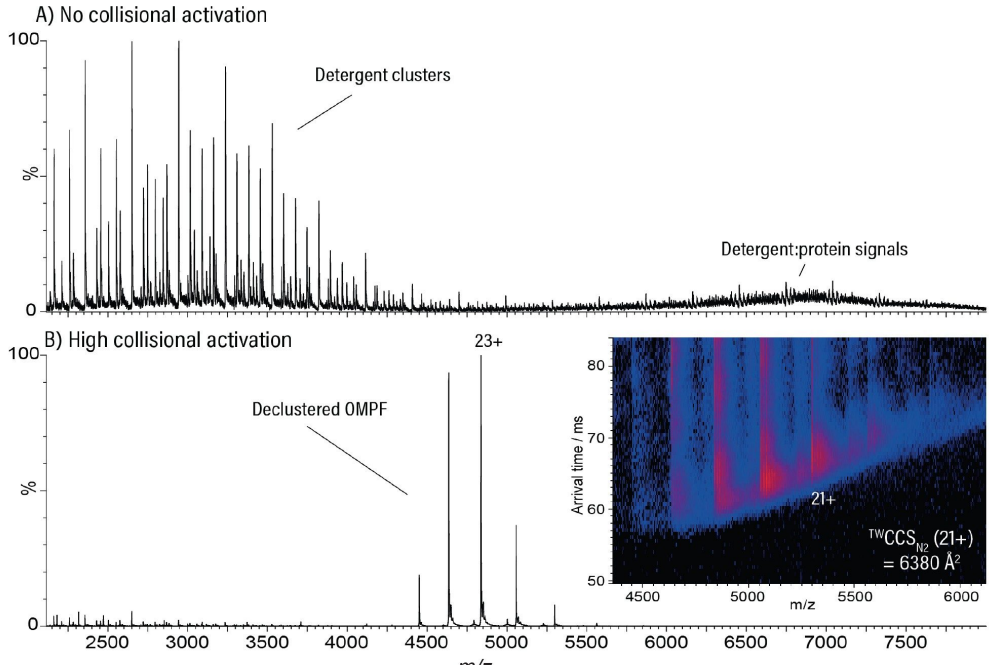


*Figure 1. Effective declustering of membrane proteins. A) Native mass spectrum of OmpF (4 µM) electrosprayed from a solution of 200 mM ammonium acetate and 1% β-OG (w/v). No additional activation voltages were applied leading to a spectrum dominated by signals attributed to detergent clusters (low m/z region) and detergent:protein complexes (high m/z region). B) Native mass spectrum with 150 V cone voltage and 200 V trap voltage to effectively liberate OmpF from the detergent micelle. A high-quality spectrum is obtained with well-resolved signals. Inset – Ion mobility mass spectrum of OmpF with activation voltages minimized to obtain CCS values of native-like folded states ($^{TW}CCS_{N2}$ (21+) = 6,380 ± 52 Å$^2$).*

Next, we applied the above established methods to investigate membrane protein-ligand binding in a drug discovery context. The protein in this case was *pf*MATE, a key model system in bacterial drug resistance studies. The protein (4 µM) was solubilized in 50 mM ammonium acetate with 0.5% C8E4 (w/v). Figure 2A shows the mass spectrum of *pf*MATE under optimum declustering settings (160 V cone, 6 V trap) with well-resolved signals obtained. Indeed, at this molecular weight (measured at 50.5 kDa) the protein signals exhibit isotopic resolution (Figure 2A, inset), with a measured resolving power (Rp) of 70,000 full-width half maximum (FWHM) in the 'V' optic mode of operation. As for OmpF above, we used the ion mobility capability of the instrument to monitor the

gas phase unfolding of *pf*MATE whilst optimizing the activation voltages to obtain the folded structure. Doing this we could determine the CCS of folded *pf*MATE (10+) to be 3,655 ± 20 Å$^2$.

Finally, *pf*MATE was incubated with a solution containing a mixture of 133 drug-like compounds taken from a high-throughput screening library. The native mass spectrum (Figure 2B) exhibited an additional series of signals with a mass 372 Da greater than the protein alone, indicating a ligand with a mass of 372 Da bound with a stoichiometry of 1:1. This detected mass could be cross-checked with the expected compounds within the library to give a good indication of the identity of the hit. With this capability, an effective ranking of drug-like or fragment-based binders could be envisioned by virtue of the ratio of bound to unbound protein directly from the native mass spectrum.

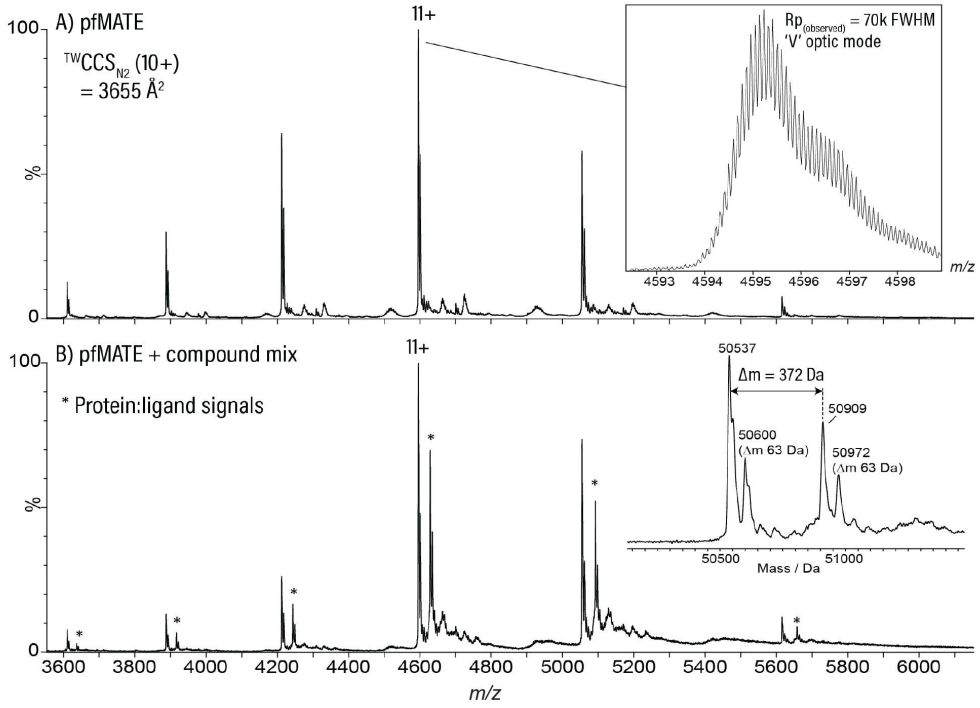


*Figure 2. Small molecule binding to pfMATE. A) pfMATE alone. High quality data are obtained at high resolution (inset). B) PfMATE after incubation with 133 drug-like molecules. Asterisks indicate signals attributed to ligand binding. Inset – MaxEnt1 deconvoluted spectrum showing a mass shift of 372 Da, the mass of the only bound ligand from the compound mix.*

## Conclusion

In this application brief we demonstrate the utility of the SELECT SERIES Cyclic IMS Mass Spectrometer for the study of membrane proteins. The collisional activation capability of the system allows for effective removal of detergent micelles required for protein solubilization to yield high quality, well-resolved mass spectral signals. Furthermore, optimization of the collision voltages enables the determination of CCS values for native-like folded states of membrane proteins by cyclic ion mobility, yielding insights into higher order structure to enable tracking of structural changes. We applied the native membrane protein methods to a study of *pf*MATE and its binding to drug-like molecules, showing how the SELECT SERIES Cyclic IMS instrument can be used to enhance drug-discovery workflows.

## References


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