# Developing a declustering ion guide and Cyclic IMS-enabled Wideband Enhancement for native top-down studies

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## INTRODUCTION

- Native top-down protein analysis is advancing the characterisation of proteoforms in a biological context
- Spectral resolution is often limited by incomplete desolvation of ions from endogenous matrices, salts, and solubilizing detergents [1,2]
- High quality tandem mass spectra remain challenging to obtain for low abundance proteoforms and large oligomeric assemblies[3]
- Here, a declustering ion guide improves protein resolution, and Wideband Enhancement (WBE) boosts tandem MS sensitivity
- NISTmAb was used to develop methods and optimize declustering, and the declustering guide is shown to enable pseudo-MS$^3$ experiments for top-down sequencing of streptavidin.
- Furthermore, we show the guide to facilitate the release of bacteriorhodopsin from a detergent micelle to enable characterization of bound lipids

## METHODOLOGY

### Samples

- NISTmAb standard (Pierce)
- Streptavidin (Pierce, 21125)
- Bacteriorhodopsin (bR) from *halobacterium salinarum* (Sigma-Aldrich)

Samples were electrosprayed from 200 mM ammonium acetate using 2 µm internal diameter borosilicate glass nanocapillaries (WPI LLC, FL, USA). To prepare native bacteriorhodopsin, lyophilized samples were resuspended, vortexed and buffer exchanged in 40 mM β-D-glucopyranoside (OG) detergent (Sigma-Aldrich) and 200 mM ammonium acetate.

### Mass Spectrometry

All experiments were performed with a Waters™ Cyclic™ IMS P20 Mass Spectrometer operating in positive nESI mode, equipped with a Dynamic Field Declustering (DFD) device (*Figure 1*). Data were acquired in MassLynx™ v4.2 Software, processed using the top-down workflow in waters_connect™ UNIFI™ software.

## REFERENCES

1. Cheng D, Guo Y, Lyu J, et al. Advances and challenges in preparing membrane proteins for native mass spectrometry. *Biotechnology Advances*. 2025;78:108483.
2. Tamara S, den Boer MA, Heck AJR. High-Resolution Native Mass Spectrometry. *Chem Rev*. 2022;122(8):7269-7326.
3. Hoang C, Uritboonthai W, Hoang L, et al. Tandem Mass Spectrometry across Platforms. *Anal Chem*. 2024;96(14):5478-5488.
4. Inada M, Kinoshita M, Matsumori N. Archaeal Glycolipid S-TGA-1 Is Crucial for Trimer Formation and Photocycle Activity of Bacteriorhodopsin. *ACS Chem Biol*. 2020;15(1):197-204.

## ACKNOWLEDGEMENTS & STATEMENTS

**Conflict of Interest statement:** All authors are employees of Waters Corporation who manufacture and sell the products highlighted in this work.

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## TECHNOLOGY

### Dynamic Field Declustering

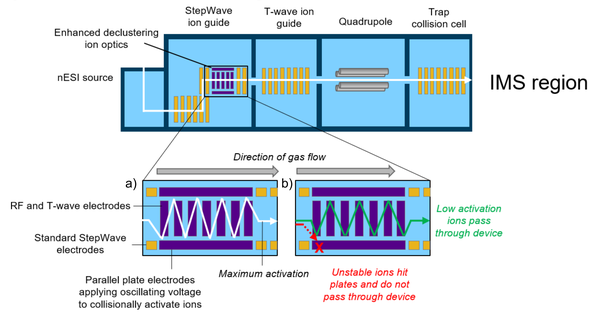
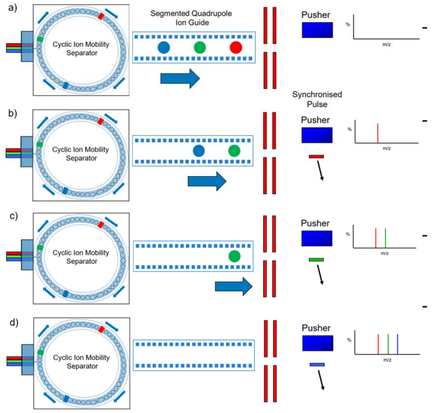



**Figure 1.** Schematic of the DFD device. Pre-IMS region is shown **(TOP),** highlighting the location of the declustering ion guide. **a)** Demonstrating ion movement with maximum activation, whereby the longest distance is travelled. **b)** Highlighting how ions are separated by different paths: unstable pathway, and low activation pathway, which continue into the quadrupole and beyond for detection.

### Wideband Enhancement

In Time of Flight (TOF) operation, incomplete transmission from a continuous beam limits sensitivity; WBE increases duty cycle by synchronising ion release with mobility-separated ion packets, improving sampling efficiency across a broad mass range.




- Ions enter IMS region and are separated by size, charge and shape.
- Ions exit the IMS region as distinct packets which arrive in the pusher region in ascending *m/z*.
- The ion packets are synchronized with the TOF push.
- This results in a drastically increased duty cycle for cyclic IMS experiments.

**Figure 2.** Schematic of WBE mode of operation and the corresponding spectra produced

## RESULTS

### Optimizing declustering with NISTmAb

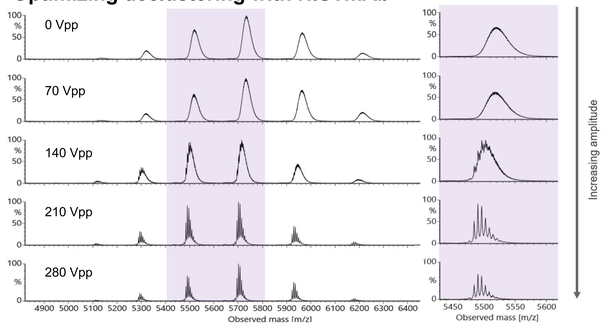



**Figure 3.** Nanoelectrospray ionization spectra for NISTmAb antibody at increasing DFD amplitudes and fixed frequency (60 kHz). Increasing amplitudes from 0-280 Vpp improved declustering and apparent peak resolution of NISTmAb glycoforms, demonstrating that DFD can simply and efficiently desolvate and decluster native samples with minimal tuning required.

### Top-Down MS of Streptavidin

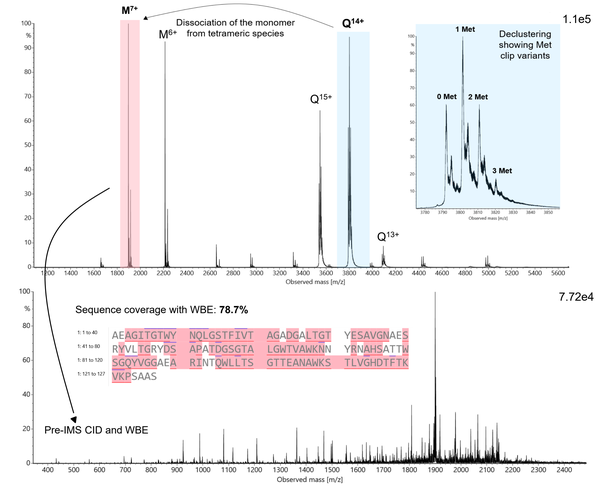



**Figure 4.** Top-down MS of native streptavidin. **(TOP)** DFD set at an amplitude of 175 Vpp and a 60 kHz frequency declustered the tetramer (Q), improving apparent peak resolution to show the Met clip variants *(blue),* in addition to partially dissociating the 7+ monomer (M) *(red)*. **(BOTTOM)** The monomer was quadrupole selected and fragmented by CID in a pseudo-MS$^3$ experiment for top-down analysis, using WBE to produce a high-quality spectrum, achieving 78.7% coverage.

### Native bacteriorhodopsin

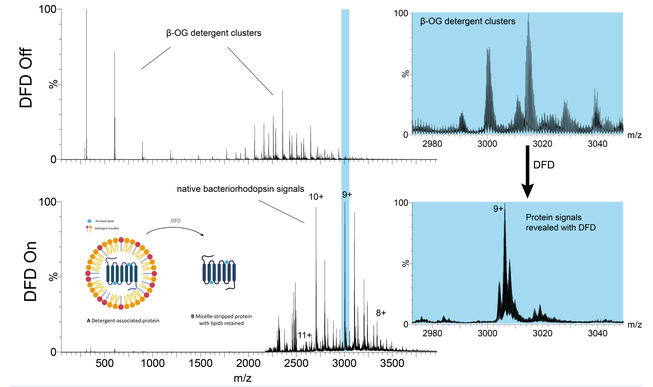



**Figure 5.** Utilizing DFD to study native bR releases the protein from the micelle to reveal protein signals on the native mass spectra. The blue inset highlights the 9+ charge state of monomeric native bR, demonstrating how DFD effectively declusters the protein signals from detergent to reveal the isotopically resolved monomer.

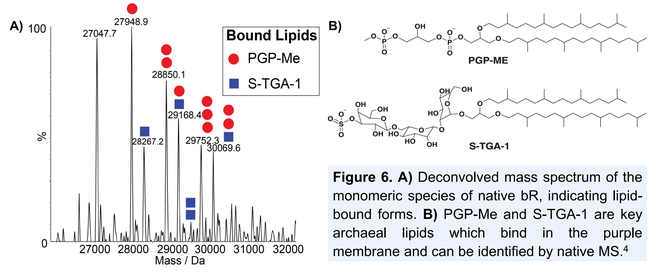



**Figure 6. A)** Deconvolved mass spectrum of the monomeric species of native bR, indicating lipid-bound forms. **B)** PGP-Me and S-TGA-1 are key archaeal lipids which bind in the purple membrane and can be identified by native MS.[4]

## CONCLUSIONS & FUTURE WORK

**Conclusions:**

- **DFD** streamlines native MS by efficiently removing salt/solvent adducts and by releasing membrane proteins from detergent micelles.
- **WBE** increases sensitivity to deliver high quality top-down data in less time.
- In combination with DFD, WBE enables pseudo-MS$^3$ workflows, yielding sequence level information directly from native complexes.

**Future work:**

- This work establishes an enhanced analytical framework for native proteins, providing a robust platform for continued investigation.
- Ongoing studies focus on bacteriorhodopsin, with development of sample preparation strategies to support trimer detection and enable top-down analysis of the native complex