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High Throughput Native MS With Robust Ion Source Operation For The Analysis Of Proteins And Protein Complexes

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Introduction

Native mode proteins and protein complexes are typically analyzed using nanospray techniques or at capillary LC flow rates with gentle ionization conditions to achieve best responses and preserve the native conformations. However, a few recent publications suggest that higher flow LC/MS techniques perform acceptably well for native mode protein and protein complexes[1]. The current work was undertaken to examine this feasibility in detail.

Experimental

Samples

Protein standards were obtained from MilliporeSigma and used as received. Typical protein concentration was 20 µM based on the molecular weight of the protein or protein complex, dissolved in 200 mM ammonium acetate.

Software

MassHunter versions 10 and 9.1 software were used for 6545XT AdvanceBio LC/Q-TOF and IM-QTOF acquisition control, respectively. MassHunter data processing software version 10 was used throughout (Qualitative Analysis, Quantitative Analysis, BioConfirm, IM-QTOF). For some processing, UniDec deconvolution software was also used[2,3].

LC: 1260 Infinity II BioInert LC or 1290 Infinity II UHPLC, with 6-port valve and isocratic pump

Column flow was diverted to waste after the protein eluted to minimize fouling of the ion source by salts and low MW species. The isocratic pump was used to maintain flow to the Q-TOF during this time[1].

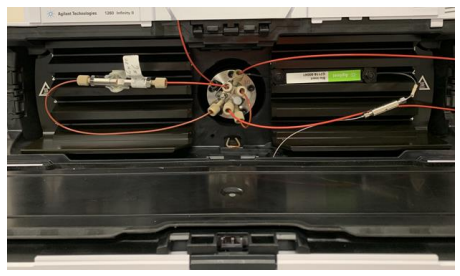

Figure 1. 10-port valve installed in column compartment, emulating a 6-port valve for diverting the salts and low MW species to waste.

Experimental

LC:

Parameter	Value
Column	AdvanceBio SEC guard column, 4.6 x 30 mm, 1.9 µm, 200 Å (PL1580-1201)
Mobile phase (both pumps)	200 mM ammonium acetate
Flow rate	0.1 mL/min
Column to waste at:	3.8 minutes
Column temp:	30 °C
Stop time	6.0 minutes
Injection volume	1.0 µL

Parameter	Value
Nebulizer pressure	60 psig
Nozzle voltage	2000 V
Capillary voltage	5500 V
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min
Drying gas temperature	350 °C
Drying gas flow	12 L/min

MS: 6545XT AdvanceBio LC/Q-TOF or 6560 IM-QTOF

Parameter	Value
Fragmentor	250 V
Skimmer (6545XT Q-TOF only)	90 V
Quad AMU setting	400 or 700
Trap RF (IM-QTOF only)	200 V
Collision energy	0 V
Mass range	*m/z* 90-10,000 or *m/z* 790-14,100
Acquisition rate	0.5 spectra/sec

Source parameter optimization using yeast alcohol dehydrogenase (ADH) tetramer

Repetitive injections of ADH tetramer were made, varying source parameters to locate the optimum response. The highest signals were obtained with high gas temperatures and flows.

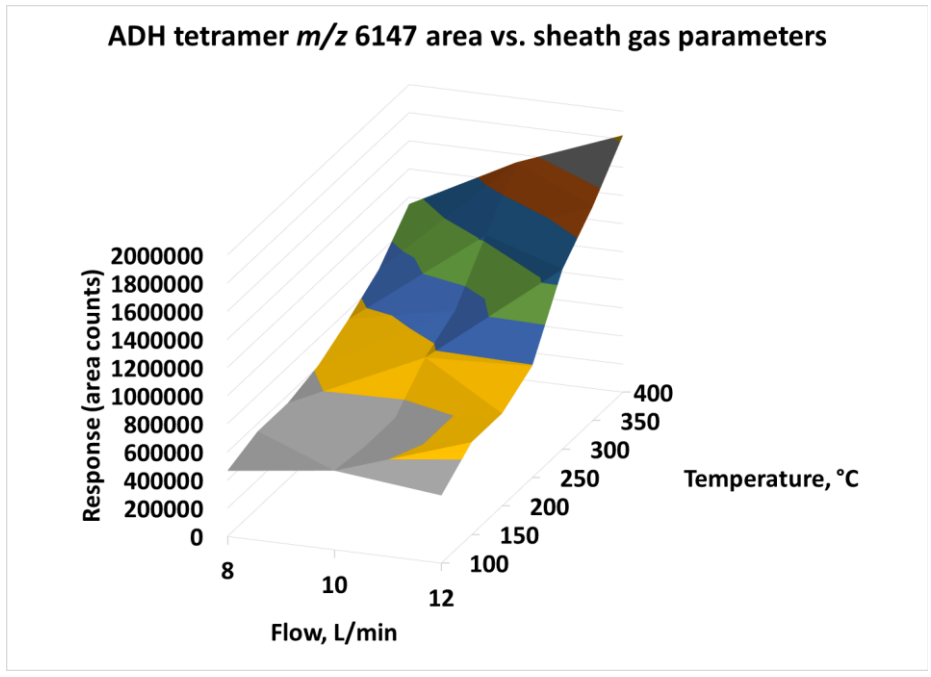

Figure 2. Example of the response of ADH tetramer (*m/z* 6147 area, 24+ charge state) to sheath gas parameters temperature and flow.

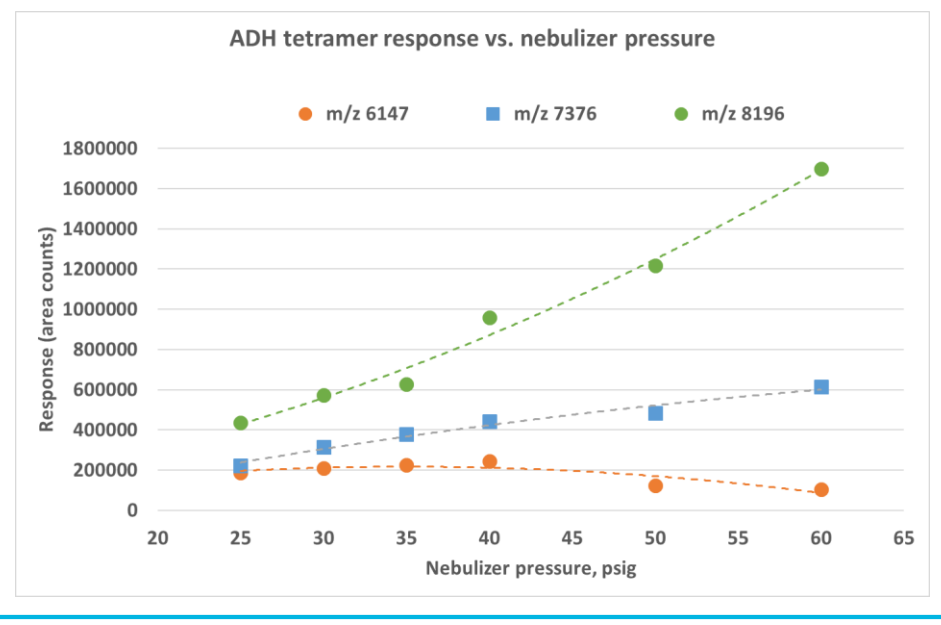

Figure 3. Response of ADH tetramer *m/z* 6147 (24+),*m/z* 7376 (20+), *m/z* 8196 (18+) to nebulizer pressure. Overall response increased with increasing nebulizer pressure.

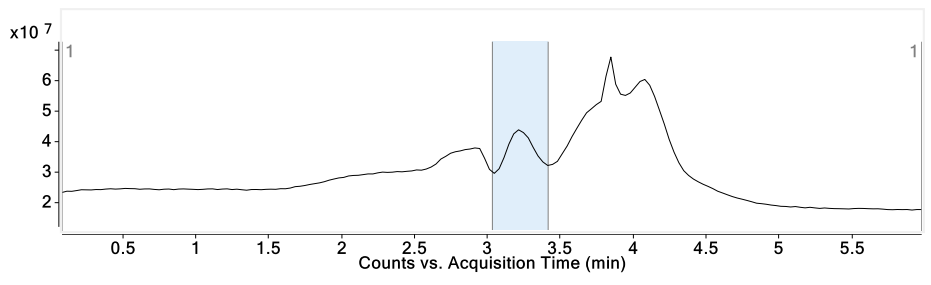

Figure 4. TIC of ADH tetramer (elution region highlighted). The large response at the end of the analysis was due to small MW singly-charged species.

ADH tetramer (6545XT AdvanceBio LC/Q-TOF)

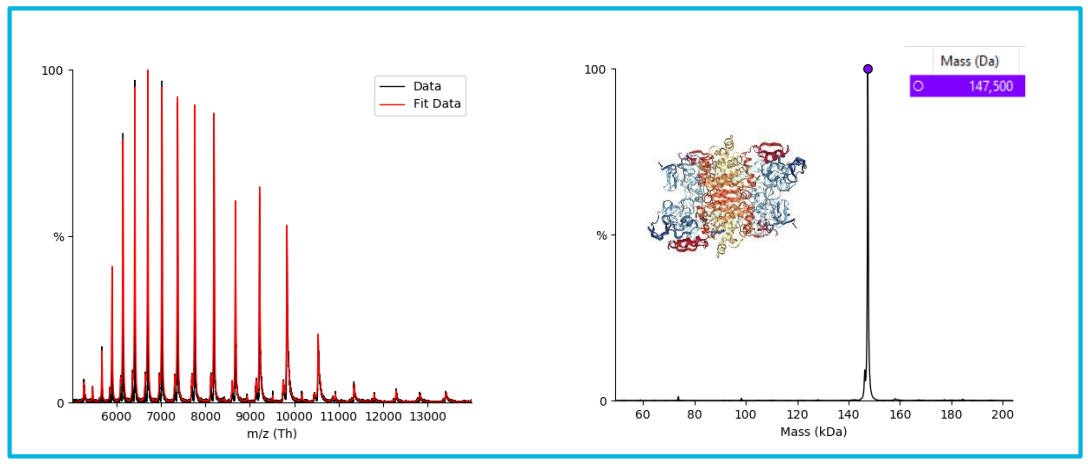

Figure 5. ADH tetramer (3 µg on-column) spectrum and deconvoluted results (expected MW 147.5 kDa). An extended charge state envelope (~26+ to 14+) was detected, more extensive than when using nanospray[4]. The cause is currently under investigation.

β-Galactosidase tetramer

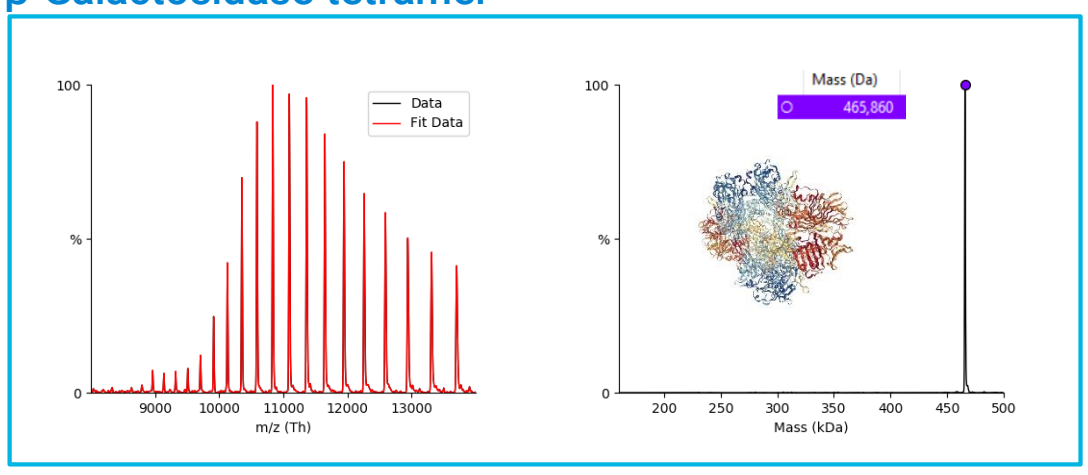

Figure 6. β-galactosidase tetramer (9 µg)spectrum and deconvoluted results (expected MW 465 kDa).

NIST mAb

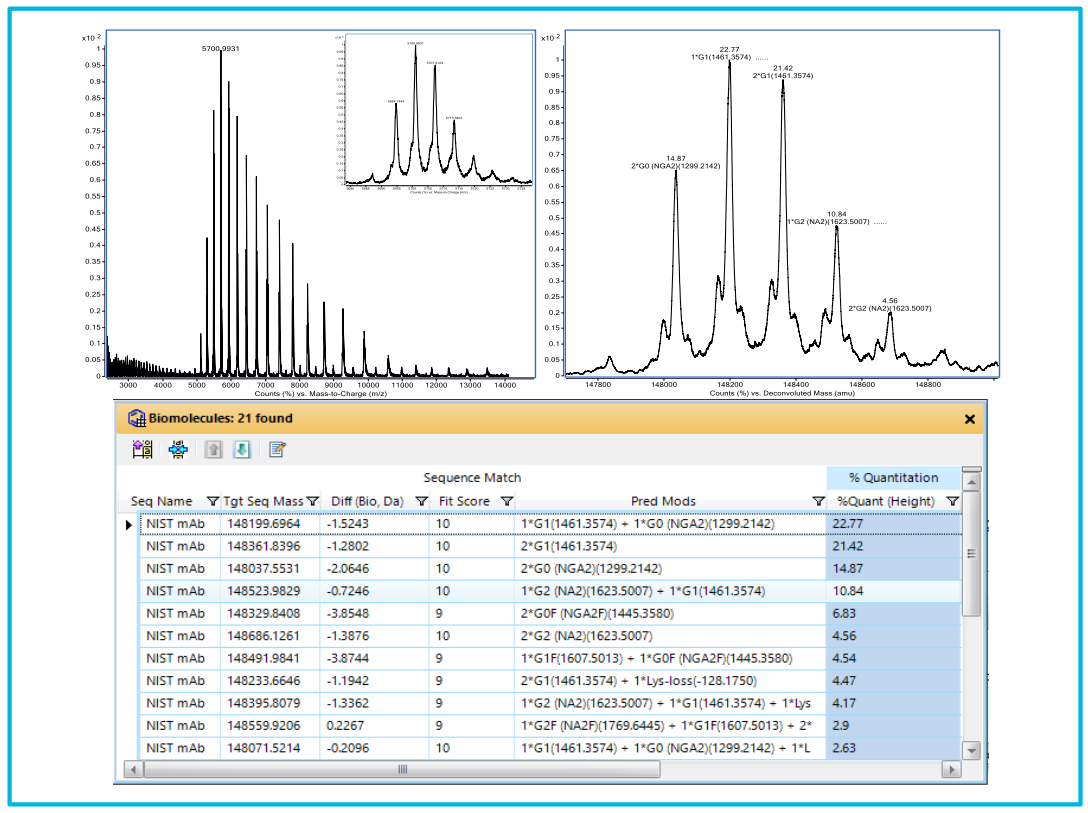

Figure 7. NIST mAb (3 µg on-column) spectrum and deconvoluted results. Denaturation appeared to be minimal (peaks *m/z* 2500-4500). Several known modifications were identified (zoom view and table).

Results and Discussion

ADH tetramer (6560 IM-QTOF)

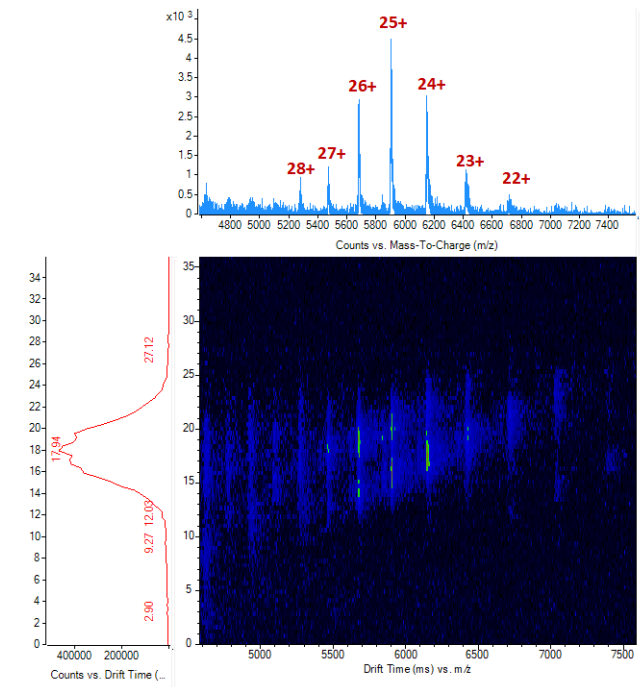

Figure 8. ADH tetramer by ion mobility Q-TOF, showing a spectrum with charge state assignments (top) and a full drift spectrum (left). Two species with overlapping charge states were apparent.

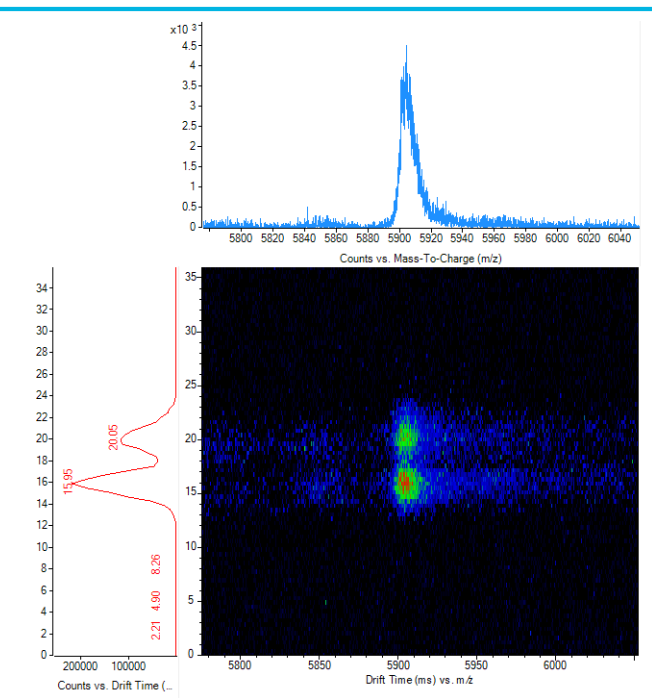

Figure 9. ADH tetramer showing charge state 25+ with two conformers.

The same ion source parameters were used on both instruments to obtain the ADH tetramer spectra shown in Figures 5 and 8. It is conjectured that the differences in charge state distribution may be due to different internal instrument/method parameters, different conformations or structures[5] resulting from different sample preparations,… The cause of the differences is currently under investigation.

Conclusions

- Robust, routine analysis of protein and protein complexes in the native mode
- One set of ion source parameters was used throughout (though further optimization likely will improve the response for some species)
- Unattended operation, 6 minutes per sample
- An extended charge state envelope was present for many proteins/protein complexes

References

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[4]Schachner, L. F., Ives, A. N., McGee, J. P., Melani, R. D., Kafader, J. O., Compton, P. D., Patrie, S. M., Kelleher, N. L.: Standard Proteoforms and Their Complexes for Native Mass Spectrometry J. Am. Soc. Mass Spectrom. **30**, 1190-1198 (2019) DOI: 10.1007/s13361-019-02191-w.

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