ENHANCED TOP-DOWN MASS SPECTROMETRY PERFORMANCE WITH A HYBRID QUADRUPOLE-MULTIREFLECTING TIME-OF-FLIGHT SYSTEM

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OVERVIEW

- **Evaluate the performance of the SELECT SERIES™ MRT mass spectrometer for the topdown characterization of denatured and native proteins.**
- **Collect denatured topdown-MS (TD-MS) data on a series of standard proteins.**
- **Determine if the high resolving power (200,000 Rs) of the MRT will help minimize false positive identifications for TD internal fragments.**
- **Collect native MS data on zinc bound carbonic anhydrase, NIST monoclonal antibody, yeast alcohol dehydrogenase tetramer, and aldolase tetramer.**

SELECT SERIES™ MRT

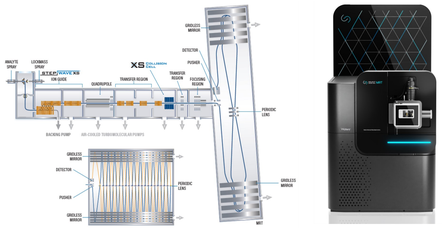

Figure 1. SELECT SERIES™ MRT instrument schematic configured with an ESI source

METHODS

- **Samples:**

Denatured TD-MS: 1.0 - 10.0 µM solutions of bovine carbonic anhydrase II, yeast alcohol dehydrogenase, and rabbit aldolase prepared in 49.5: 49.5: 1.0 water: acetonitrile: formic acid were used for denatured TD-MS experiments.

Native MS: 100 µM solutions of bovine carbonic anhydrase II, yeast alcohol dehydrogenase, rabbit aldolase, and NISTmAb were diluted to 10 µM in 200 mM ammonium acetate prior to native MS.

- **Mass Spectrometer:** SELECT SERIES™ MRT (Cooper-Shepherd et al., *J. Am. Soc. Mass Spectrom.*, **2023**)[1]

MS mode: MRT protein mode @200,000 resolving power (denatured) and Diamond protein mode @10,000 resolving power (native MS)

MS/MS method: perform broadband CID (MS^E) and MS/MS (narrow and wide quadrupole window) with a fixed collision energy (10, 15, 20, 25, 30V) applied.

METHODS

- **Inlet: ACQUITY™ I-Class UPLC™ system**

Column: ACQUITY™ BEH™ C4 Column 300Å, 1.7 µm, 2.1 x 50 mm

(PN 186004495)

Mobile Phases: MPA 0.1% Formic Acid in Water, MPB 0.1% Formic Acid in Acetonitrile

Gradient: 20-40%B over 10 minutes

Column Temperature: 80ºC

- **Data Processing:**

TD-MS mass spectra were deconvoluted using BayesSpray within the UNIFI™ topdown workflow (waters_connect™).

The deconvoluted mass spectra peak lists were uploaded into the ClipsMS (v2.0.1) computer program (Lantz et al., *J. Proteome Res.*, **2021**) for terminal and internal product ion assignment.[2]

RESULTS & DISCUSSION

CARBONIC ANHYDRASE II

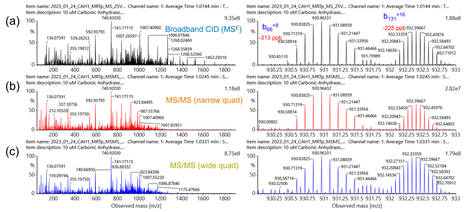

Figure 2. Denatured TD-MS fragment ion spectra of bovine carbonic anhydrase II collected with three experimental approaches: (a) broadband CID (MS^E), (b) MS/MS of the +36-charge state precursor (m/z 807.3) with a narrow quadrupole, and (c) MS/MS with a wide quadrupole. A fixed collision energy of 25V was applied in the collision cell. Right Panel: expanded view of m/z 930 – 933 to illustrate 200,000 resolving power and the mass accuracy for the b_{66}^{+8} (-313 ppb) and b_{131}^{+16} (-225 ppb) terminal fragment ions.

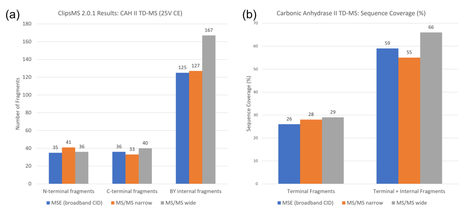

Figure 3. Panel (a) total number of N-terminal, C-terminal, and BY internal fragments identified at (± 2 ppm) for the broadband CID (MS^E), MS/MS narrow quadrupole, MS/MS wide quadrupole experimental approaches. Panel (b) resulting sequence coverage (%) for the terminal fragments and terminal + internal fragments. Note the drastic improvement in the sequence coverage when the internal BY fragments are included.

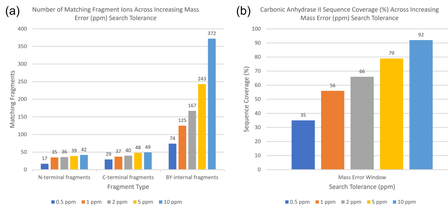

Figure 4. Panel (a) total number of N-terminal, C-terminal, and BY internal fragments identified across increasing mass error (ppm) search tolerance for the carbonic anhydrase II MS/MS wide quadrupole experiment with 25V CE applied. Panel (b) resulting combined terminal + internal fragment sequence coverage (%) for each mass error search tolerance. Note that the number of possible internal fragments matches drastically increases above the ±1 ppm mass tolerance.

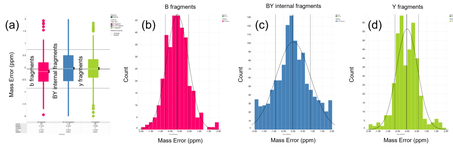

Figure 5. Panel (a): box plot containing the combined mass error distributions of the matching terminal / internal fragments from twelve TD-MS mass spectra from Carbonic Anhydrase II with three experimental approaches (broadband CID (MS^E), MS/MS (narrow), and MS/MS (wide quadrupole) with 10, 15, 20, 25V CE applied. Panels (b-d): mass error distribution plots for the B terminal fragments, BY internal fragments, and Y terminal fragments with a gaussian peak fit applied.

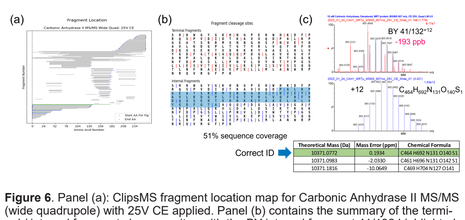

Figure 6. Panel (a): ClipsMS fragment location map for Carbonic Anhydrase II MS/MS (wide quadrupole) with 25V CE applied. Panel (b) contains the summary of the terminal / internal fragment cleavage sites with the BY internal fragment 41/132 highlighted. The combined protein sequence coverage is 51% (search tolerance ±750 ppb). Panel (c): expanded view of the +12-charge state BY internal fragment 41/132 with the corresponding theoretical isotope model. The inset table contains two additional candidate internal fragment ions that are near the deconvoluted mass of 10,371.0752 Da (-193 ppb). The Correct ID is highlighted in green.

ALDOLASE

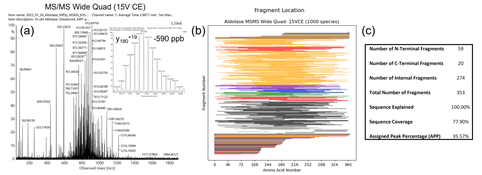

Figure 7. Panel (a): Rabbit Aldolase TD-MS experiment using MS/MS with a wide quadrupole window centered on the m/z 835.29 (+47) precursor ion with 15V collision energy applied. The inset figure shows an expanded view on the y_{180}^{+19} fragment ion with -590 ppb mass accuracy. Panel (b) ClipsMS sequence coverage map using 750 ppb search tolerance on terminal and internal fragment ions. Panel (c) summarizes the statistics from the ClipsMS results.

Modification Type	Mass Shift (Da)	Amino acid residue	Number of residues required	Color
None	0	None	0	grey
2H2O-	-36.0211	None	0	black
H2O-	-18.0106	None	0	black
2NH3-	-34.0531	None	0	black
NH3-	-17.0265	None	0	black
H-	-1.00783	C	1	red
2H-	-2.01566	C	2	green
3H-	-3.02349	C	4	blue
4H-	-4.03132	C	5	purple
1S	31.9721	C	1	orange
2S	63.9442	C	2	orange
S+	-32.9799	C	1	orange
2SH-	-65.9598	C	2	orange
S-	-31.9721	C	1	orange
2S-	-63.9442	C	2	orange
H-S+	30.9643	C	1	orange
2(H-S+)	61.9286	C	2	orange
SH2-	-33.9878	C	1	red
2(SH2-)	-67.9755	C	2	orange

Table 1. Unlocalized modifications for ClipsMS fragment matching of CAD of Aldolase and Yeast Alcohol Dehydrogenase. Non-reducing conditions peptide mapping experiments of rabbit aldolase and yeast alcohol dehydrogenase confirms a population of the protein molecule contains cysteines residues that are involved in disulfide bridging.

YEAST ALCOHOL DEHYDROGENASE

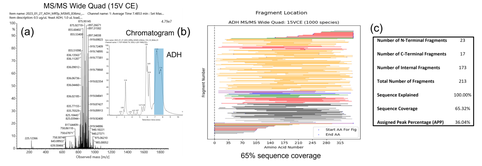

Figure 8. Panel (a): Yeast Alcohol Dehydrogenase TD-MS experiment using LC-MS/MS with a wide quadrupole window centered on the m/z 836.14 (+44) precursor ion with 15V collision energy applied. The inset figure shows the TIC chromatogram for the targeted MS/MS experiment. Panel (b) ClipsMS sequence coverage map using 750 ppb search tolerance on terminal and internal fragment ions. Panel (c) summarizes the statistics from the ClipsMS results.

NATIVE MS

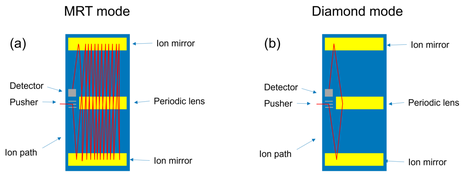

Figure 9. Description of (a) MRT and (b) Diamond mode ion beam flight path options of the SELECT SERIES™ MRT.

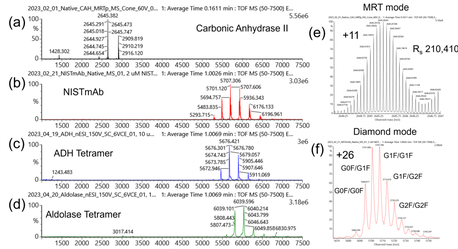

Figure 10. Native mass spectra of (a) zinc bound bovine carbonic anhydrase II collected in MRT protein mode. Panel (b) NISTmAb, (c) yeast alcohol dehydrogenase tetramer, and (d) rabbit aldolase tetramer native mass spectra collected using diamond mode. Panel (e) shows the expanded view of the $[M+11H]^{+11}$ zinc bound form of CAH II with a measured mass spectral resolving power of 210,410. Panel (f) illustrates the observed glycoform resolution achieved for of NISTmAb (+26 charge state).

CONCLUSIONS

- The denatured TD-MS experiments on the SELECT SERIES™ MRT suggests that the mass accuracy search criteria for TD fragment matching can be reduced to ±750 ppb for terminal and internal fragment identification.
- The high resolving power (200,000 Rs) can be particularly useful to distinguish between multiple candidate BY internal fragment ions.
- The Diamond mode (10,000 Rs) flight path enables large native proteins and/or protein complexes to be analyzed.

REFERENCES

1. Cooper-Shepherd, et al., *J. Am. Soc. Mass Spectrom.*, **2023** 34(2), 264-272
2. Lantz, et al., *J. Proteome Res.*, **2021**, 20, 1928-1935

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