Fragmentation of Singly-charged Peptide Derivatives Produced by Atmospheric Pressure Matrix-assisted Laser Desorption Ionization

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Overview

- Address difficulty in fragmenting singly-charged AP-MALDI peptides ions in ion trap [1,2]
- Use previously reported sulfonic acid derivatization process [3]
- Compare fragmentation patterns
  - ES MS/MS of doubly-charged species produce more fragments than ES MS/MS of singly-charged analyte
  - Derivatization improves fragmentation in singly-charged peptide derivatives by AP-MALDI MS/MS (and ES MS/MS)

Objective

- Use the sulfonic acid derivatization process [3] to make peptide ions formed by AP-MALDI easier to fragment into predominantly y-type ions in an ion trap

Introduction

- AP-MALDI tends to produce singly-charged ions, as does conventional MALDI
  - PROBLEM: Ion trap MS/MS typically utilizes fragmentation of multiply-charged ions; higher charge state increases energy of collisions in the ion trap
  - NEED: A way to better fragment AP-MALDI singly-charged ions in an ion trap
  - SOLUTION: Use sulfonic acid derivatives which would allow for easier fragmentation of the singly-charged AP-MALDI peptide ions

Methods

- Derivatize Angiotensin I (ATI), Substance P (Sub P), ASHLGLAR, and cytochrome c (cyto c) tryptic digest using chlorosulfonylacetyl chloride [3]
- Analyze peptides and peptide derivatives by ES MS/MS and AP-MALDI MS/MS on a Finnigan LCQ IT-MS to determine fragmentation patterns

Sulfonic Acid Derivative

- Normal peptide
  - H_2N-(peptide sequence)-CO_2H

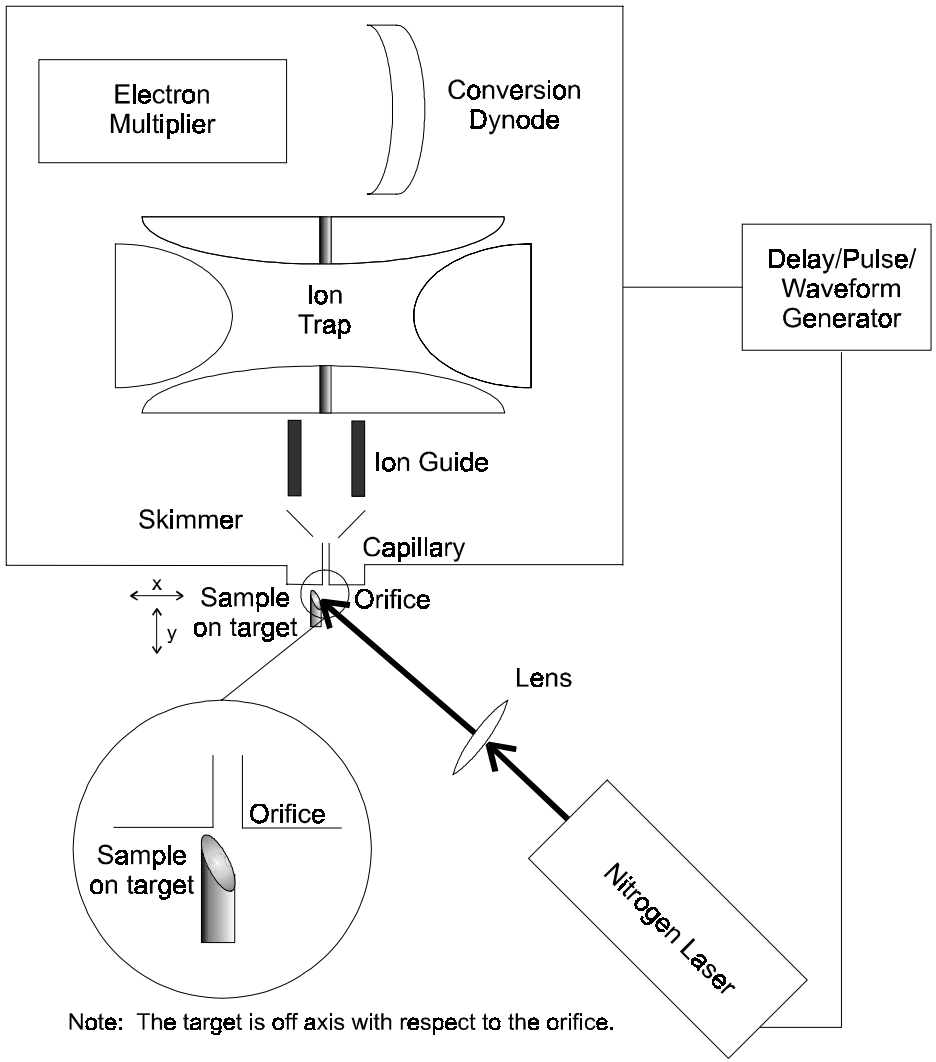
- Peptide derivative
  - $^-O_3SCH_2CONH$-(peptide sequence)-CO_2H

1) Sulfonic acid attaches to the N-terminus

2) First added proton (at most basic site, such as arginine side chain) produces neutral molecule

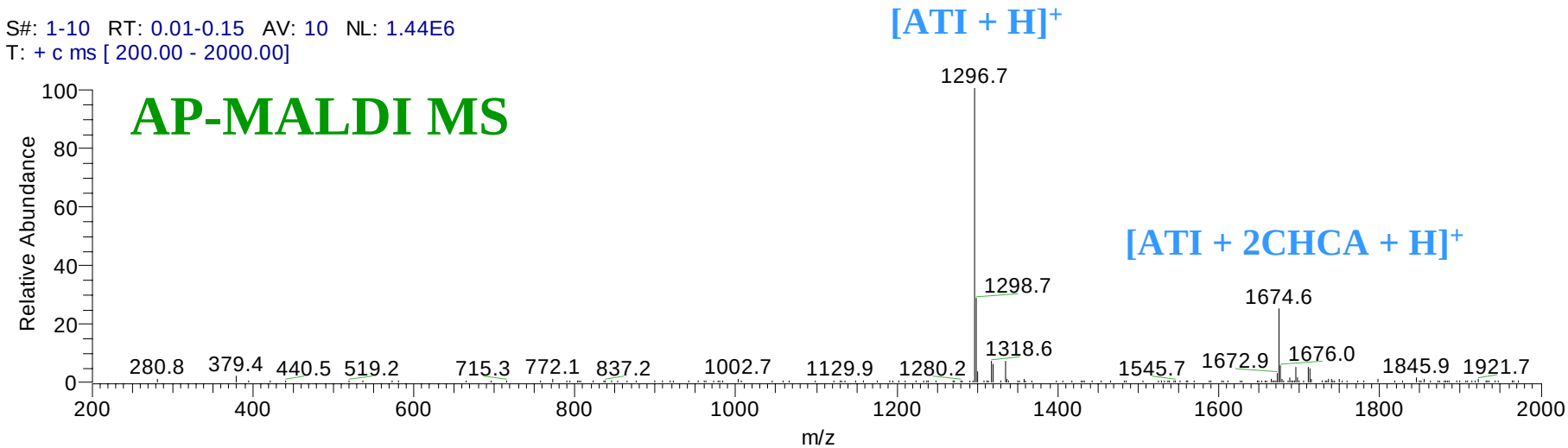
3) Second added proton (to produce +1 state) now has higher likelihood to localize along chain, inducing y-type fragmentation

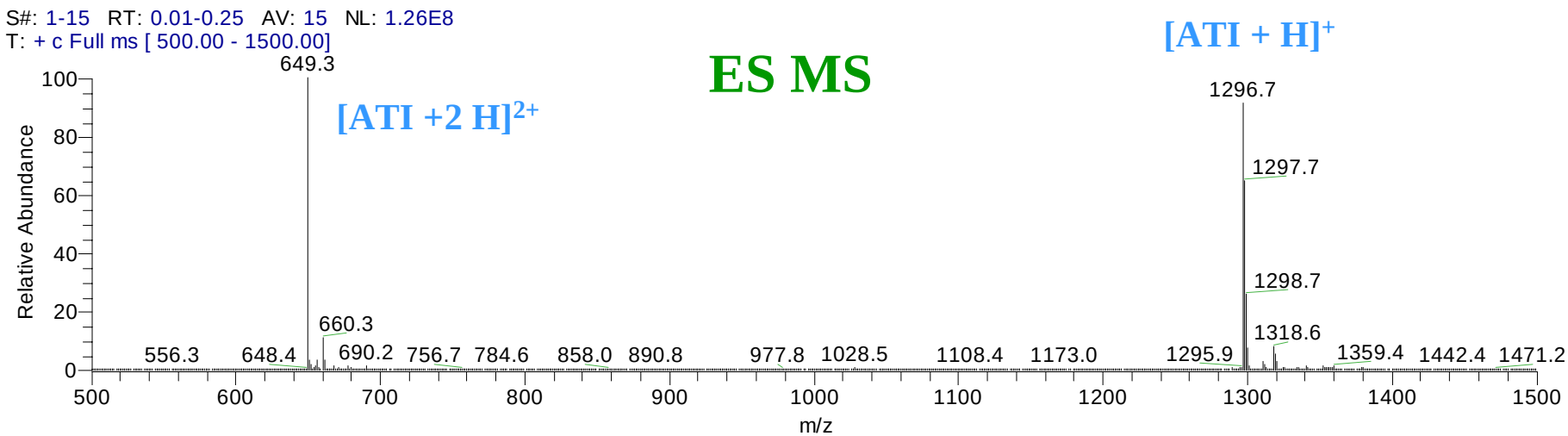
AP-MALDI IT MS



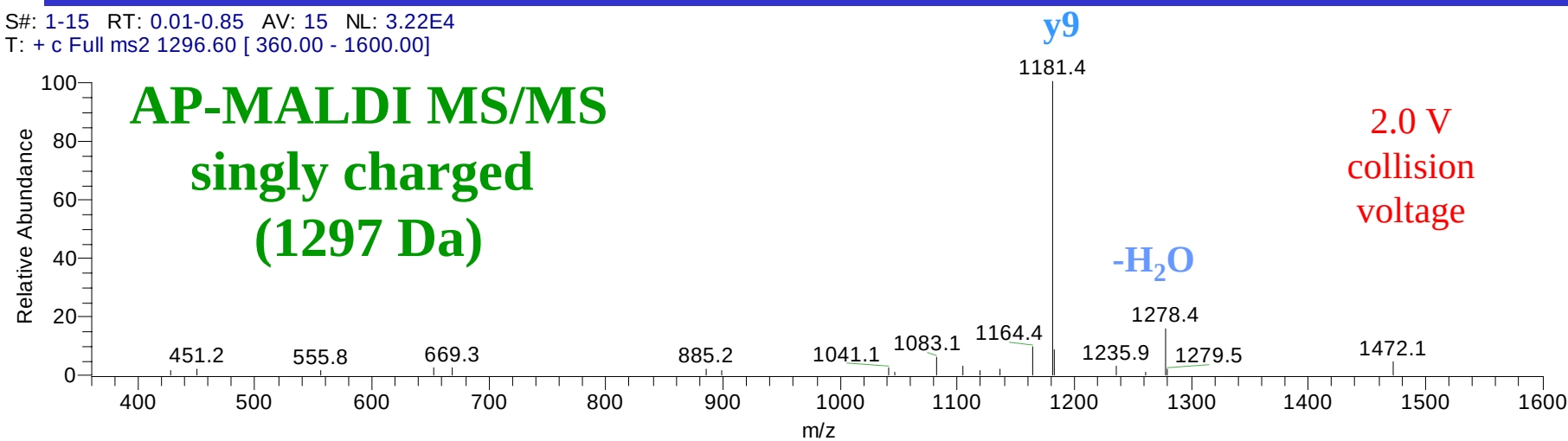
Note: The target is off axis with respect to the orifice.

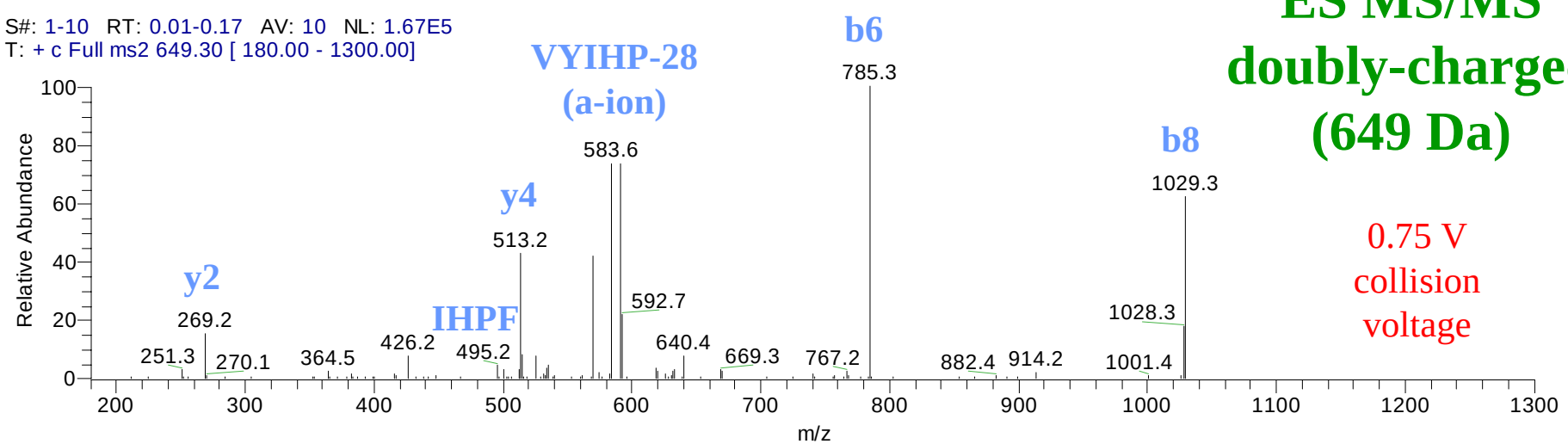
ATI Spectral Features of AP-MALDI and ESI Differ



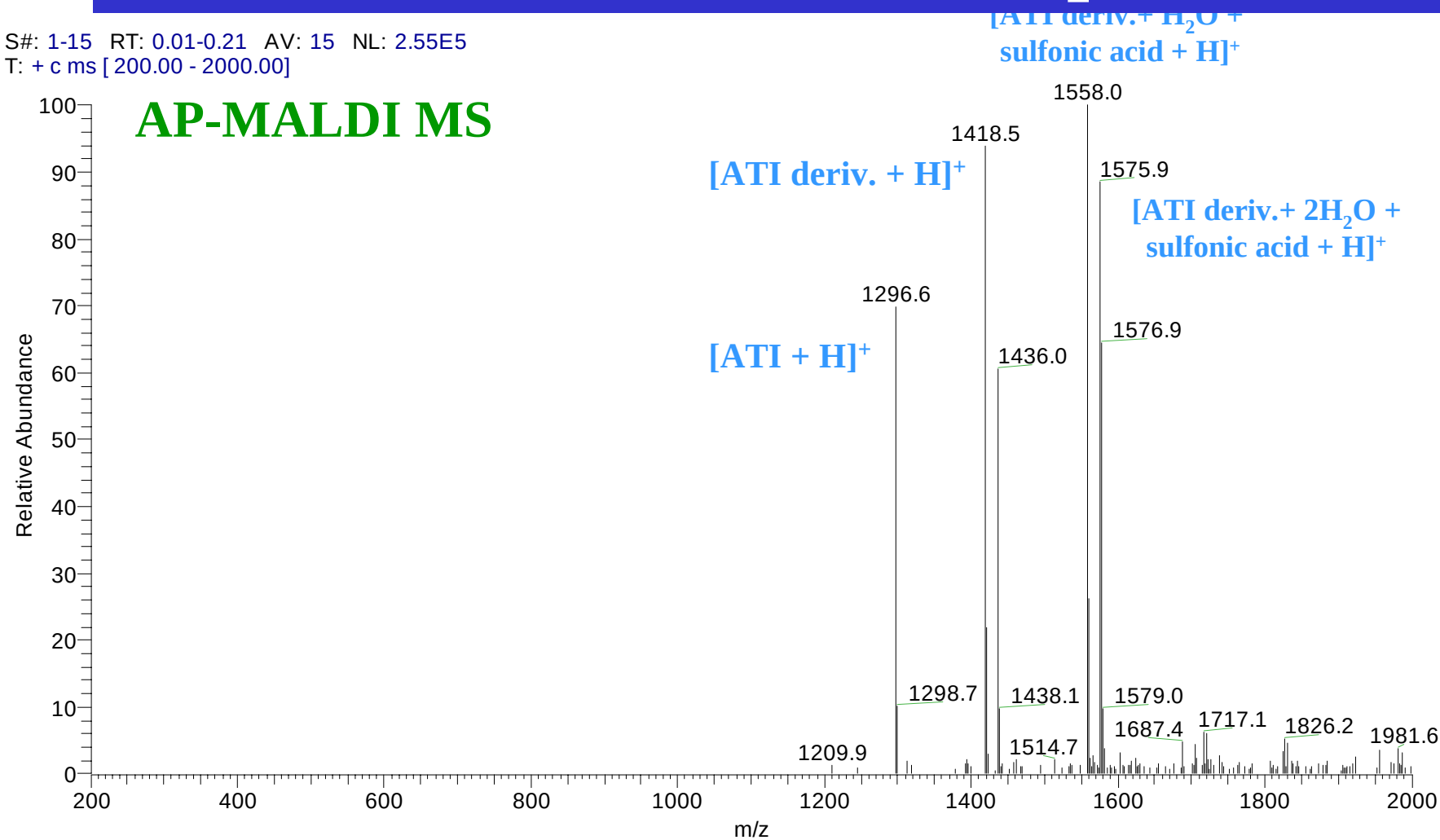


ATI Fragmentation Patterns Depend on Charge State

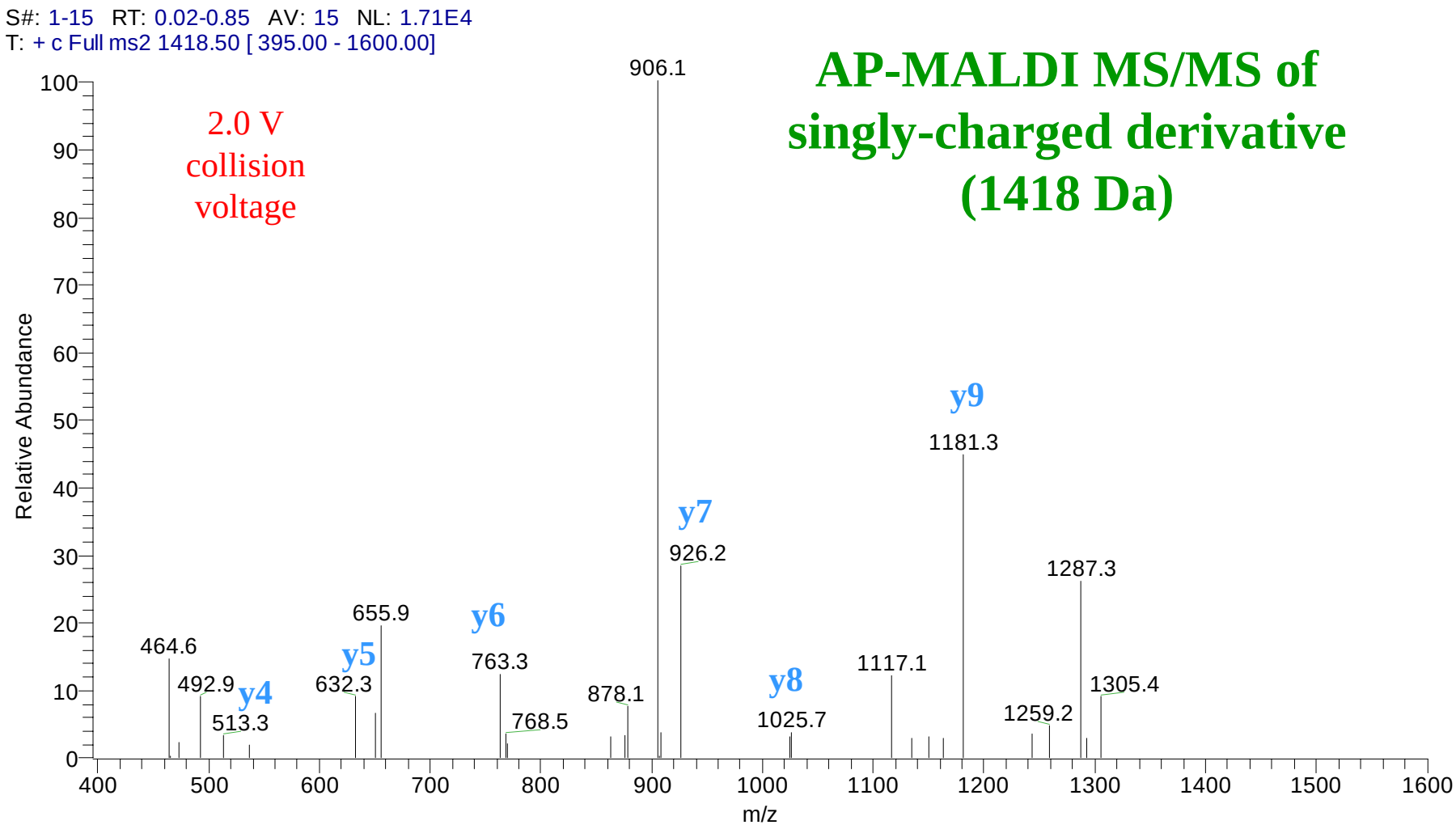




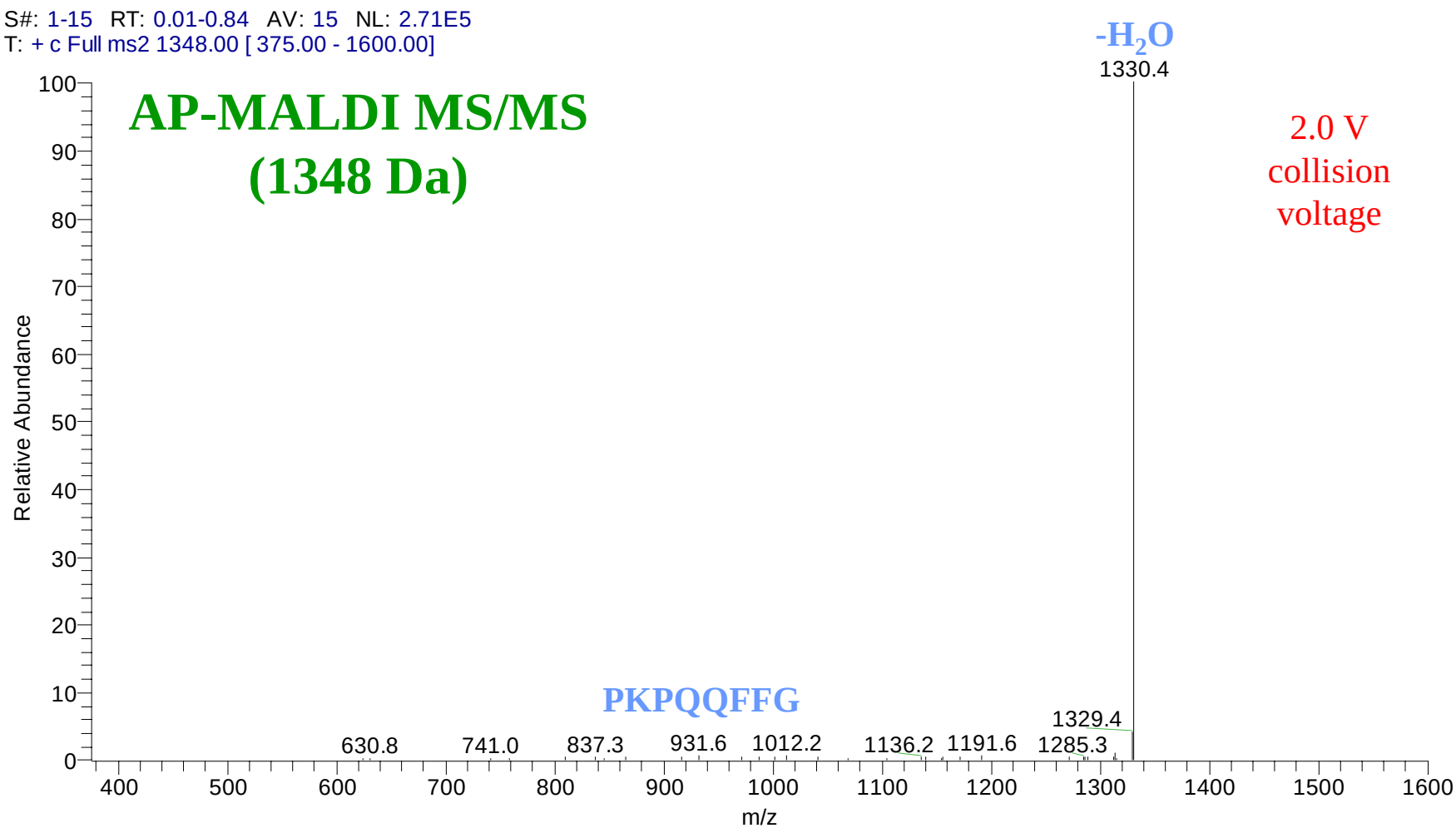
Derivatization Attaches Several Sulfonic Acid Groups



ATI Derivative Fragments into y-ions

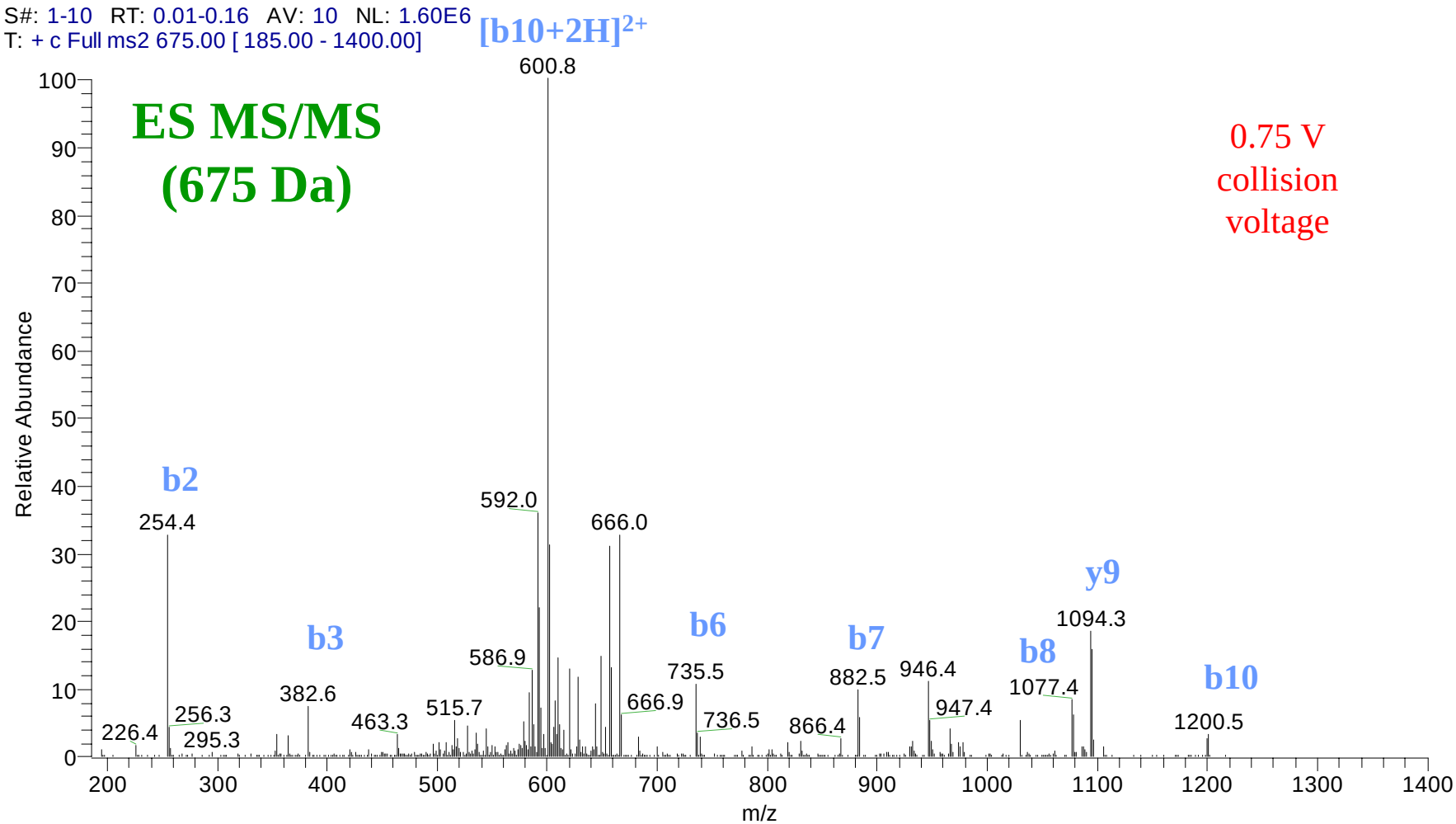


Sub P Fragments Poorly

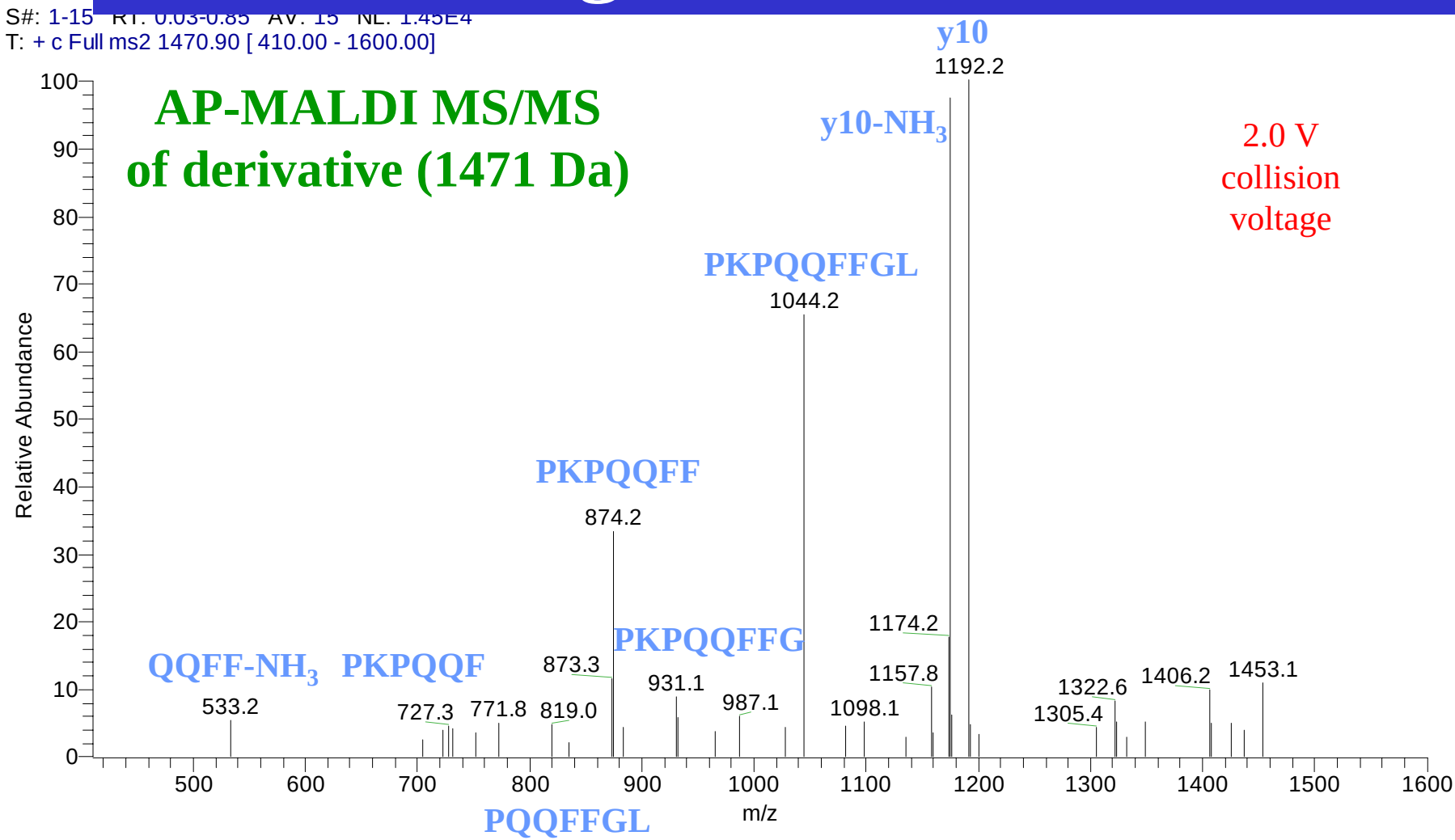


Note: amide at C-terminus on Substance P

Doubly-Charged ES Sub P Fragments Well



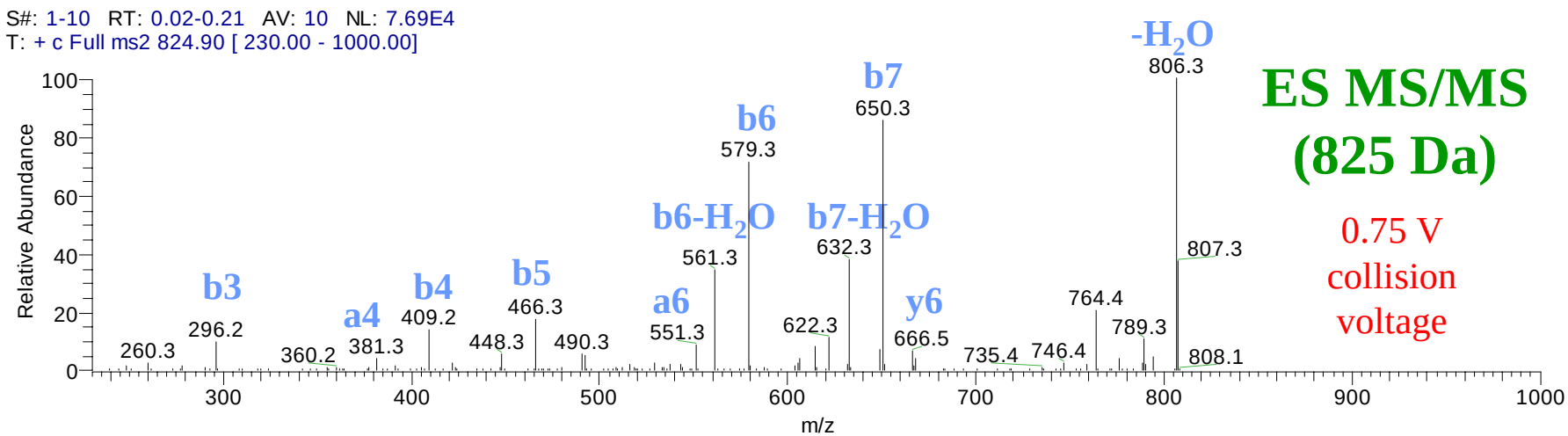
Singly-Charged Sub P Derivative Fragments Well

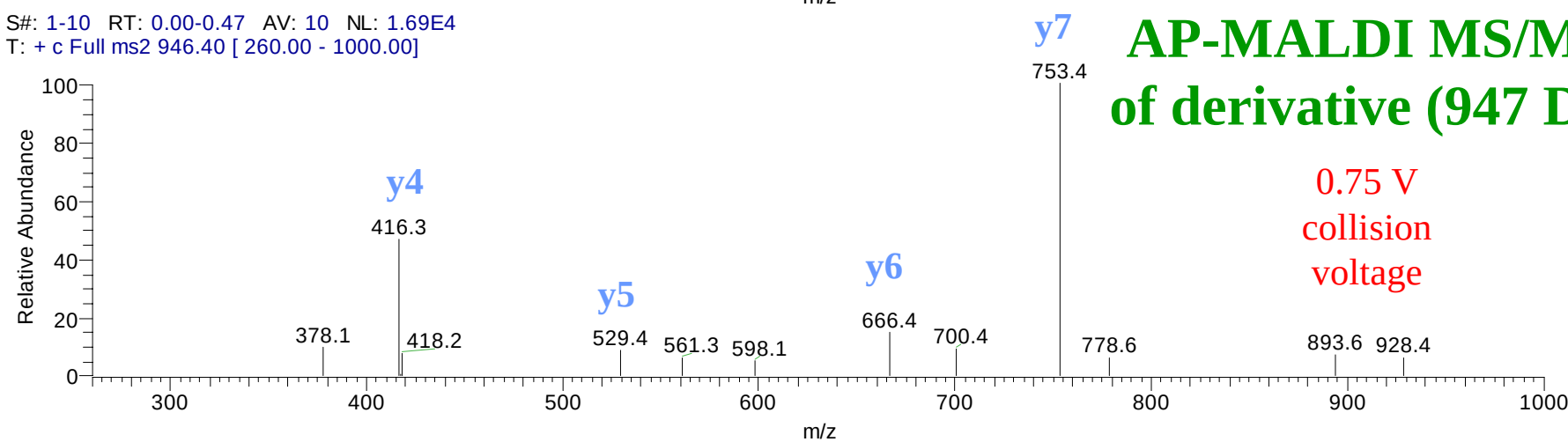


Sub P Sulfonic Acid Derivative

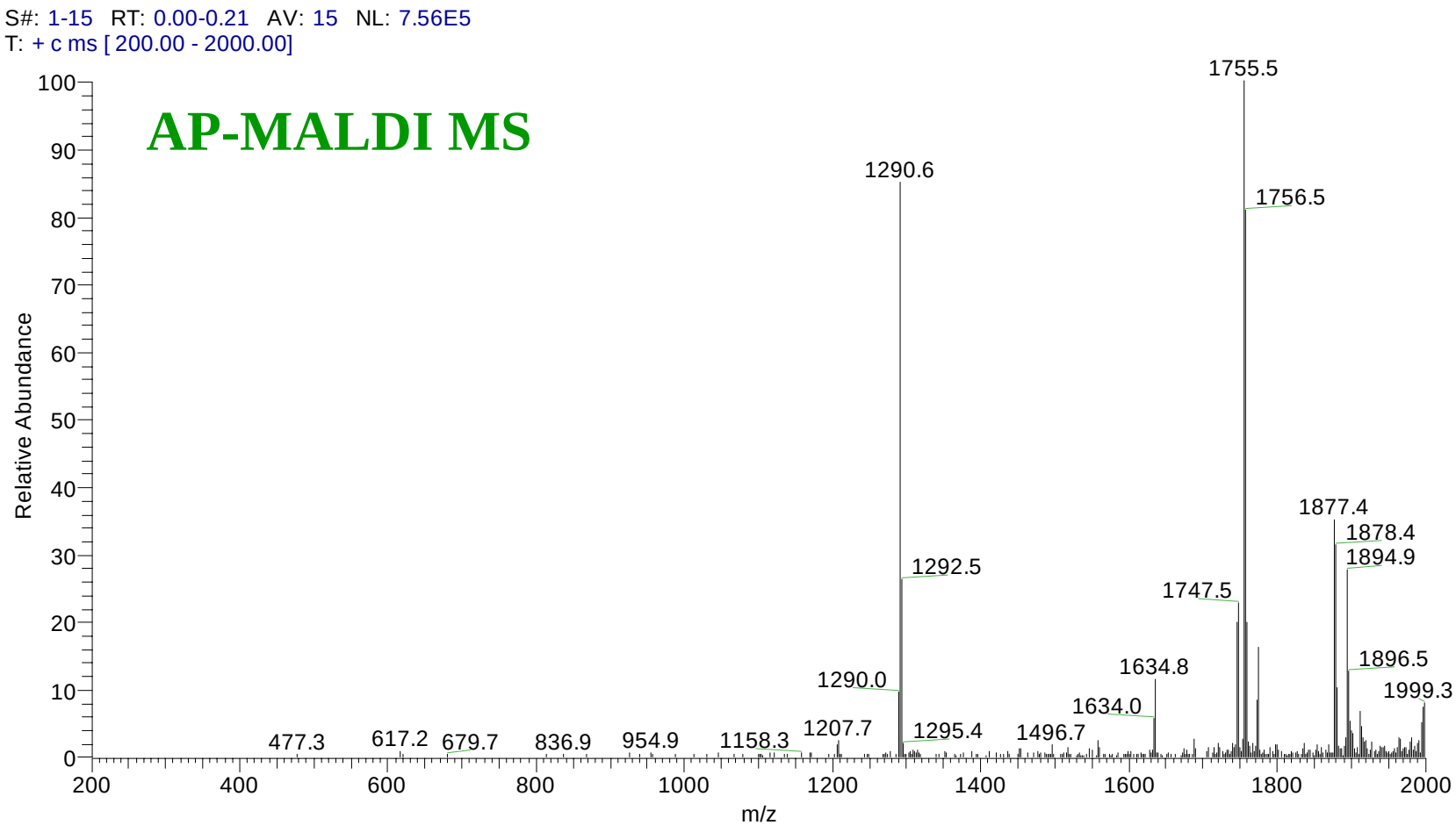
- Substance P with amide C-terminus
  - H_2N-(RPKPQQFFGLM)-$CONH_2$
- Resulting Substance P derivative
  - $^{+}H_3N$-(RPKPQQFFGLM)-$CONHOCH_2CSO_3^{-}$
  - Because of the amide, the sulfonic acid attaches to the C-terminus instead of the N-terminus.

Simpler ASHLGLAR MS/MS Spectrum from Derivative

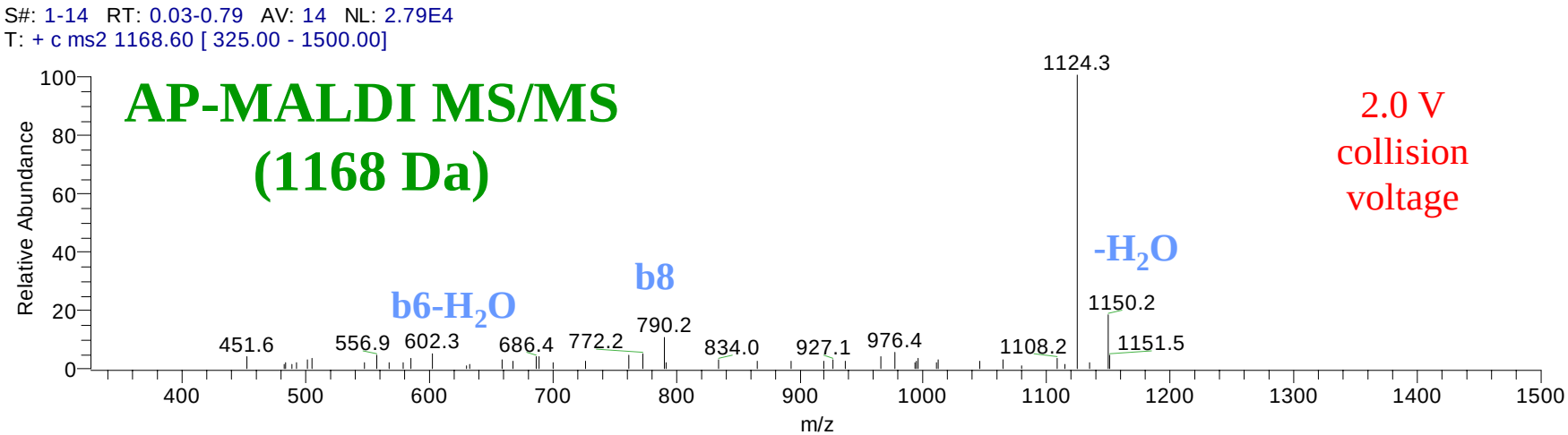


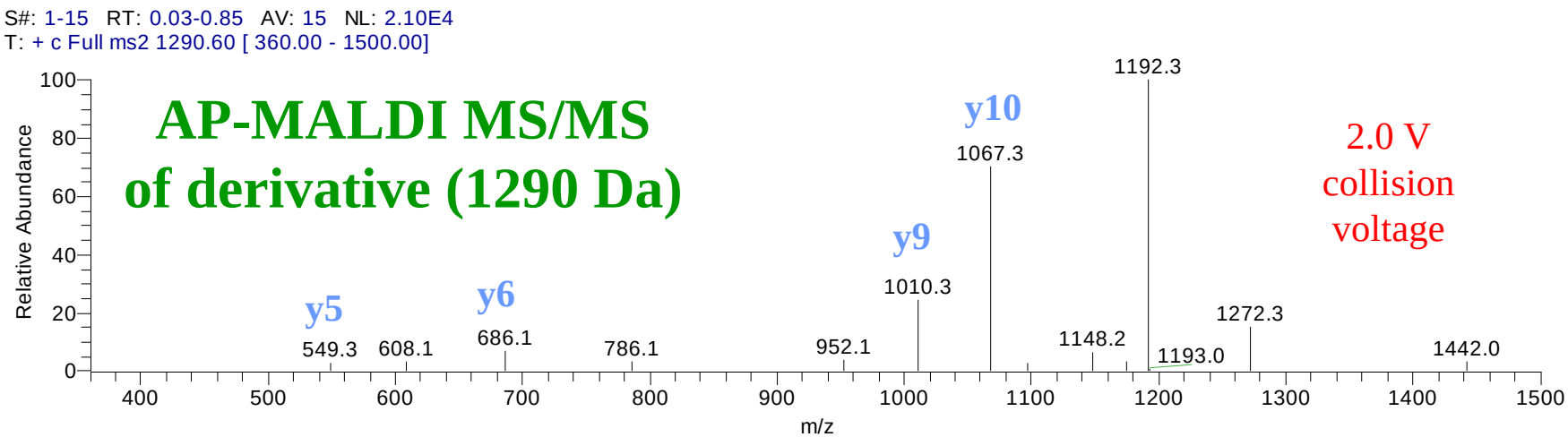


Derivatization Works for Cyto C Tryptic Digest



Derivatization Improves MS/MS of Fragment TGPNLHGLFGR





Conclusions

- As expected, ES MS/MS of doubly charged species produces more peptide mapping information than AP-MALDI MS/MS of singly charged analytes
- Singly-charged peptide derivatives show fragmentation with mostly y-ions

Conclusions

- The amide C-terminus of Substance P changes the expected fragmentation scheme
- Sulfonic acid derivatives enhance the utility of AP-MALDI MS/MS for peptides

References

(1) V.V. Laiko, M.A. Baldwin, A.L. Burlingame, *Anal. Chem.*, 2000, **72**, 652-657.

(2) V.V. Laiko, S.C. Moyer, and R.J. Cotter, *Anal. Chem.*, 2000, **72**, 5239-5243.

(3) T. Keough, R.S. Youngquist, and M.P. Lacey, *Proc. Natl. Acad. Sci. USA*, 1999, **96,** 7131-7136.

Acknowledgements

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