首届中国计算蛋白质组学研讨会

中国科学院计算技术研究所
INSTITUTE OF COMPUTING TECHNOLOGY , CHINESE ACADEMY OF SCIENCES

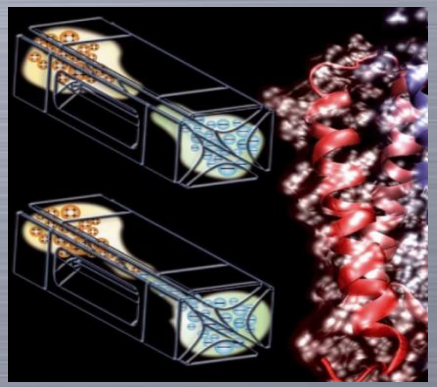
电子转运裂解质谱特征及其在蛋白质鉴定中的应用

Electron Transfer Dissociation (ETD): Characterizations and Applications in Protein Identification

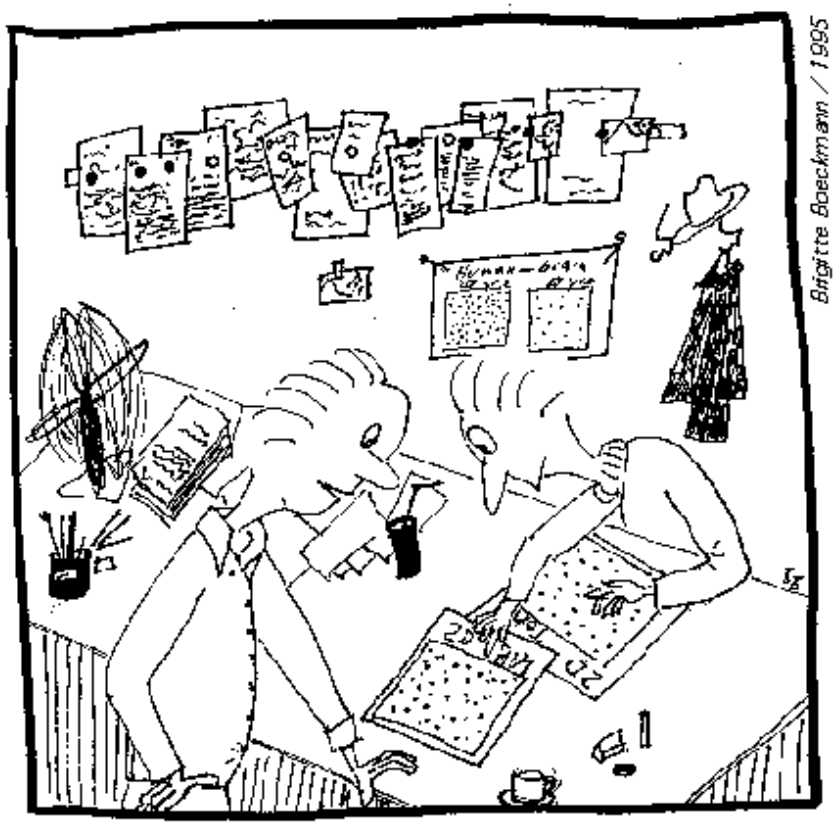
孙瑞祥

中科院计算所

2010.11.11



Puzzle: One Missing or One Extra ?

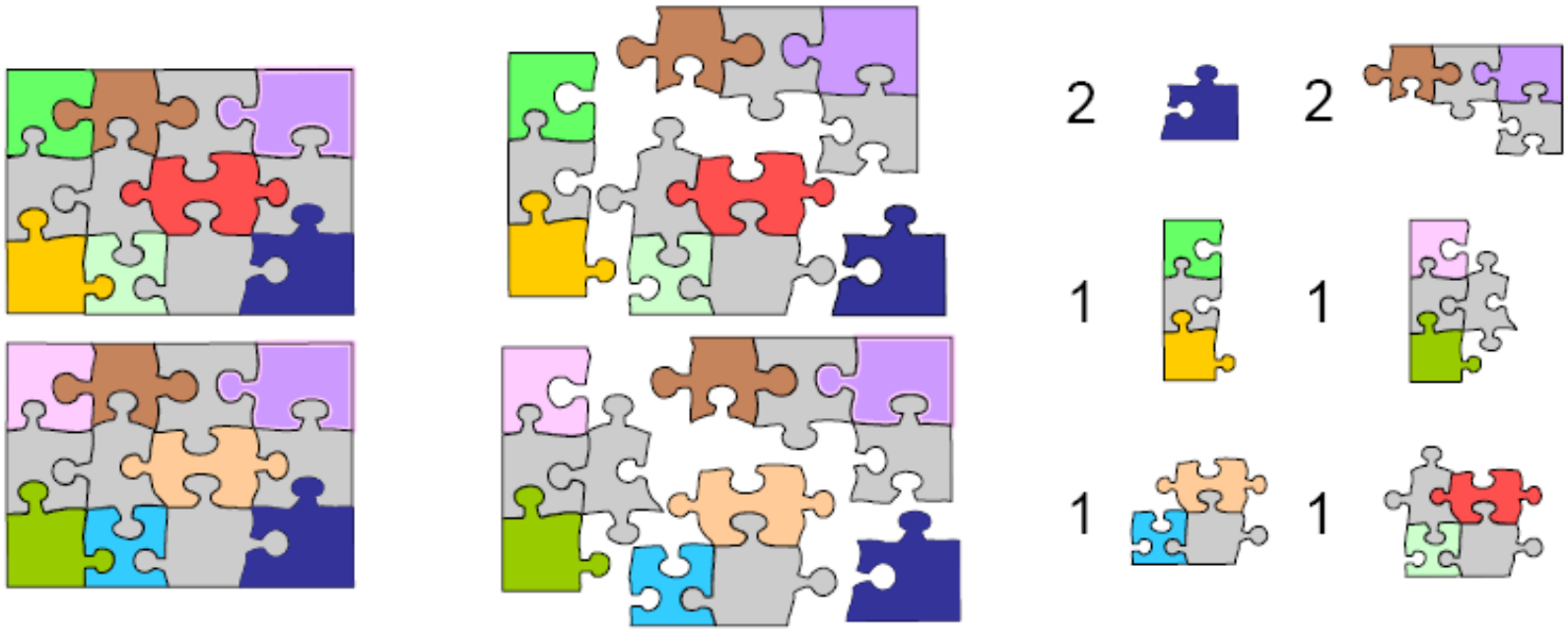


Y:
"You've got one protein missing …"

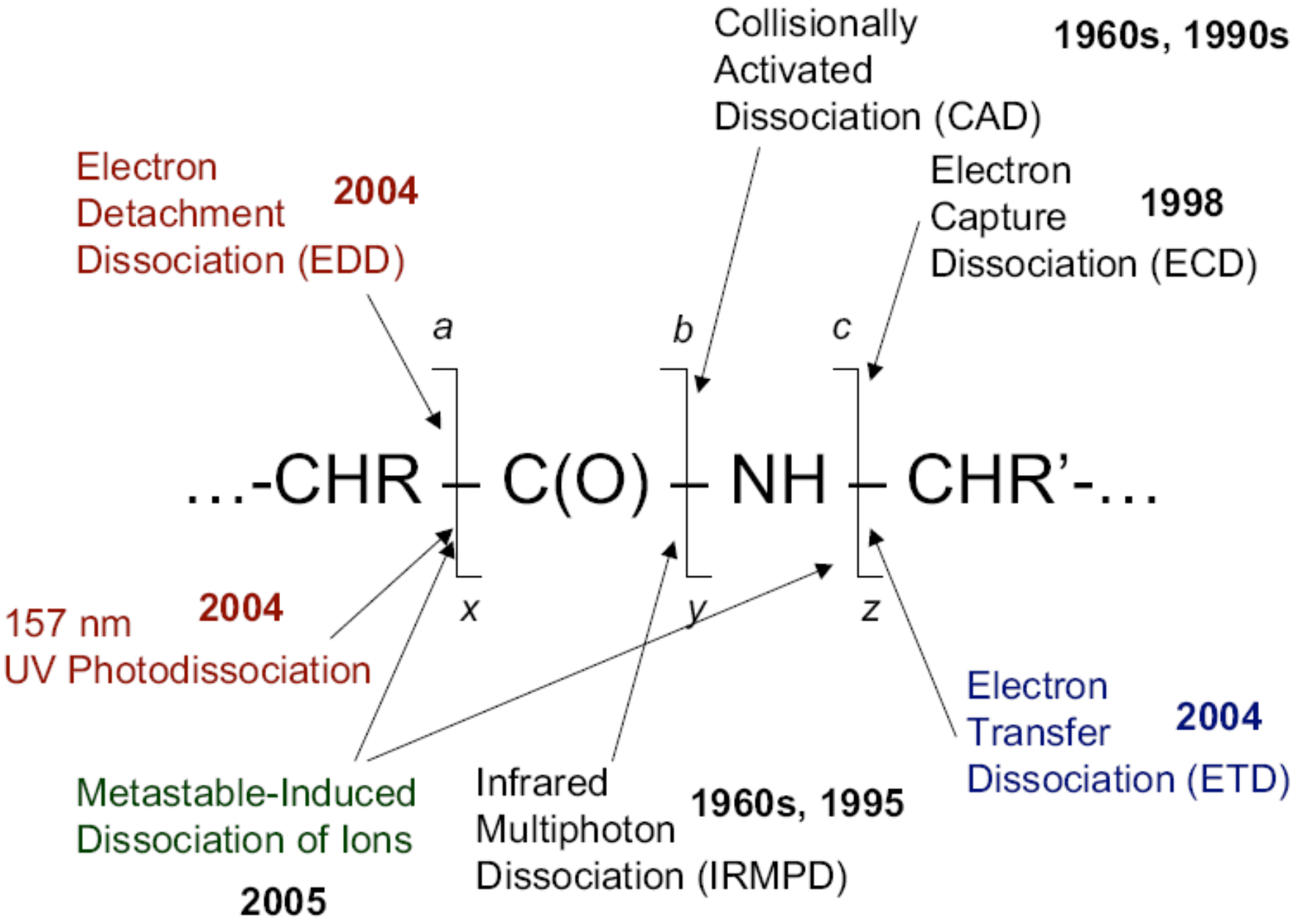
Q:
"No, you've one extra protein !"

Tandem Mass Spectrometry

MS #1	Fragmentation Chamber	MS #2
Sorting molecules	*Breaking molecules*	*Sorting Pieces*



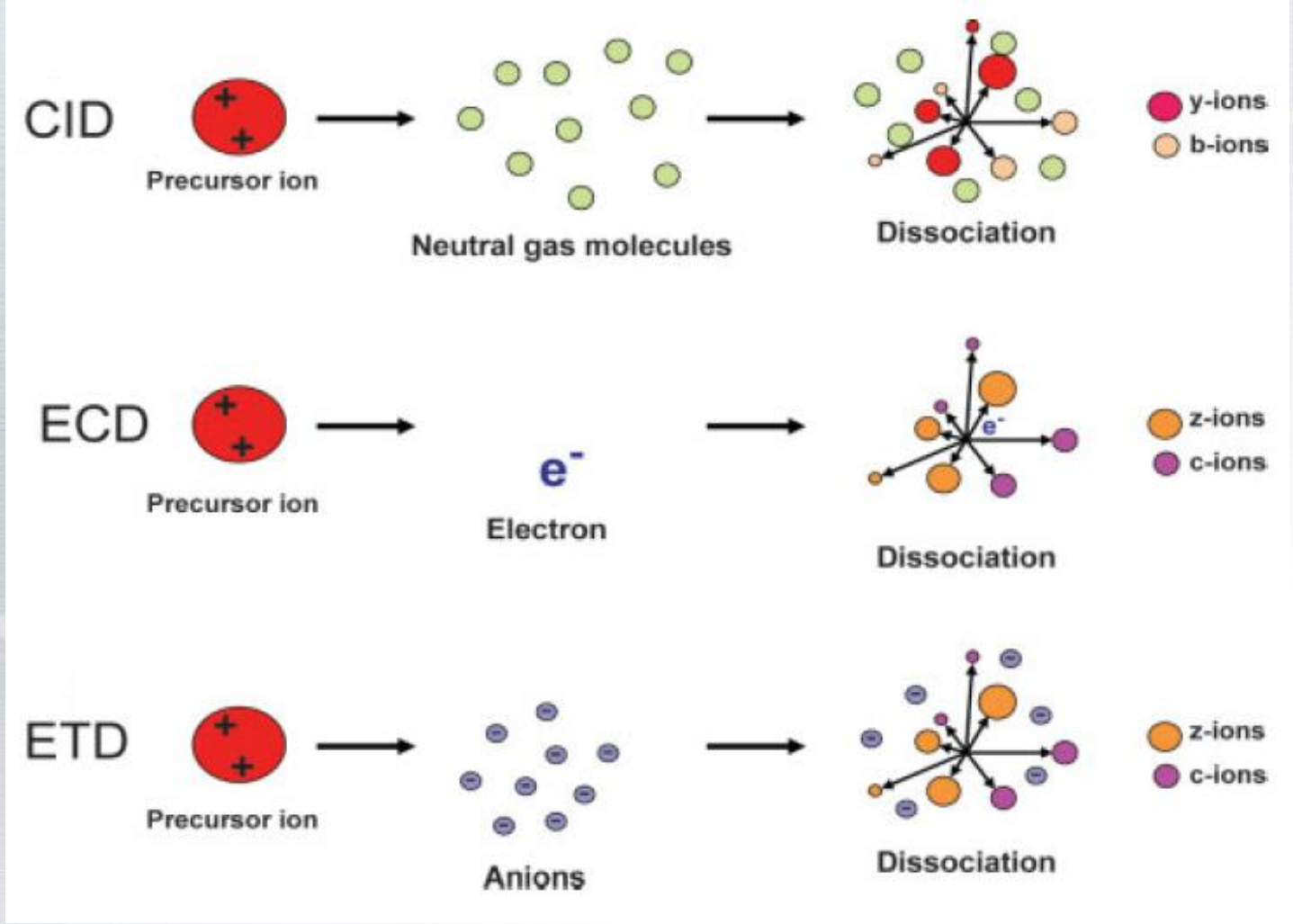
Polypeptide backbone fragmentation



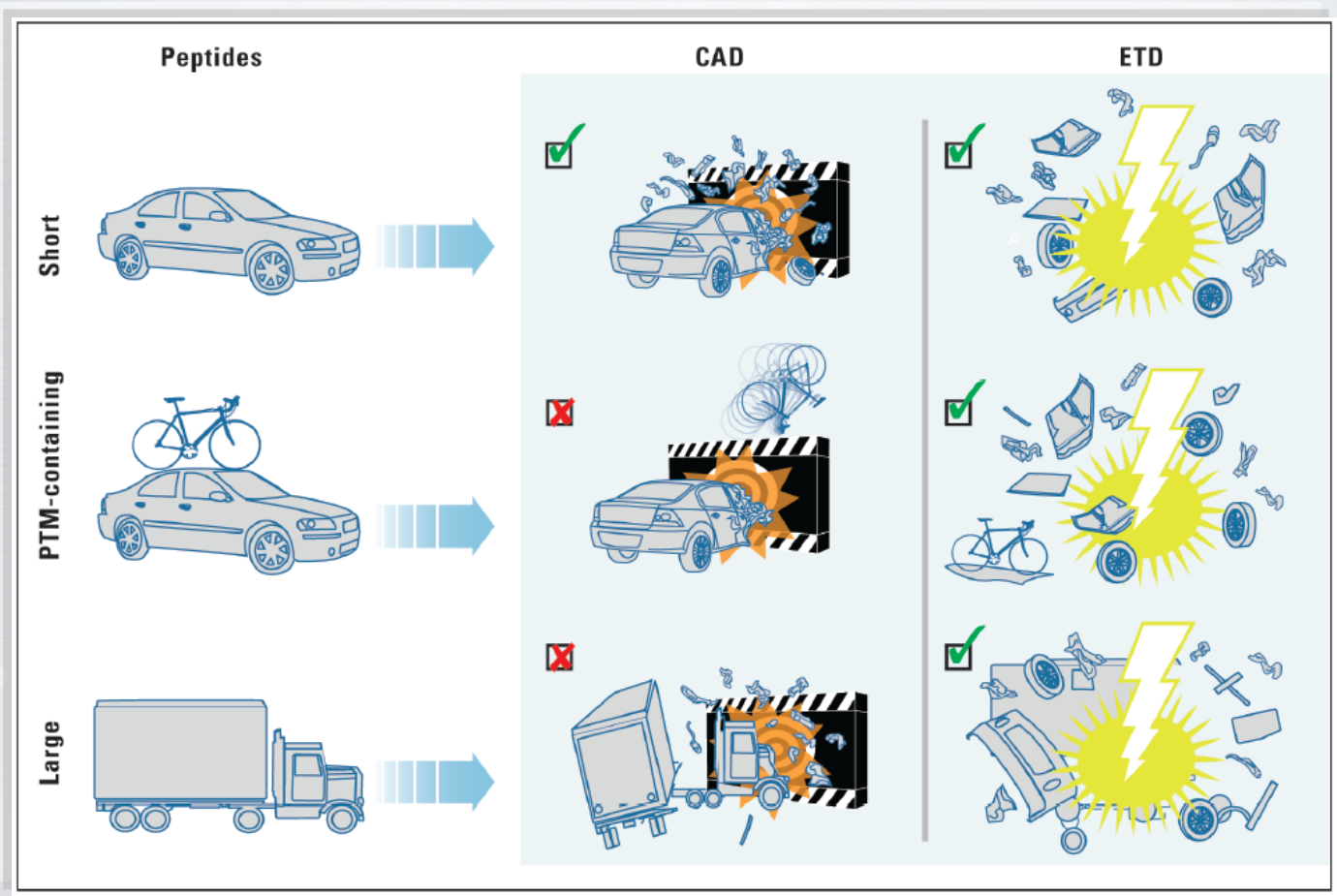
Outline

- What and Why ETD
- How to Deal with ETD Spectra
- ETD Characterizations
- Applications and Results

CID-ECD-ETD


CID
Precursor ion
Neutral gas molecules
Dissociation
y-ions
b-ions
ECD
Precursor ion
e-
Electron
Dissociation
z-ions
c-ions
ETD
Precursor ion
Anions
Dissociation
z-ions
c-ions

Peptides or Proteins: CID and ETD



Coon, J. J. (2009) Collisions or electrons? protein sequence analysis in the 21st century. Anal. Chem. 81, 3208–3215

ETD Pioneers

- Donald F. Hunt, Univ. of Virginia

- Joshua J. Coon, Univ. of Wiscosin

- John Edward P. Syka, ThermoScientific

PNAS 2004 Paper: Cited 730(2010-11-11)

Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry

John E. P. Syka*†‡, Joshua J. Coon‡§, Melanie J. Schroeder§, Jeffrey Shabanowitz§, and Donald F. Hunt§¶‖

*Engineering Physics Program and §Department of Chemistry, University of Virginia, Charlottesville, VA 22901; †Thermo Electron, San Jose, CA 95134; and ¶Department of Pathology, Health Sciences Center, University of Virginia, Charlottesville, VA 22908

Edited by Fred W. McLafferty, Cornell University, Ithaca, NY, and approved May 17, 2004 (received for review April 15, 2004)

Peptide sequence analysis using a combination of gas-phase ion/ion chemistry and tandem mass spectrometry (MS/MS) is demonstrated. Singly charged anthracene anions transfer an electron to multiply protonated peptides in a radio frequency quadrupole linear ion trap (QLT) and induce fragmentation of the peptide backbone along pathways that are analogous to those observed in electron capture dissociation. Modifications to the QLT that enable this ion/ion chemistry are presented, and automated acquisition of high-quality, single-scan electron transfer dissociation MS/MS spectra of phosphopeptides separated by nanoflow HPLC is described.

electron capture dissociation | fragmentation | ion/ion reactions | charge transfer | ion trap

Six years ago, McLafferty and coworkers (1) introduced a unique method for peptide/protein ion fragmentation: electron capture dissociation (ECD). In this method, multiply protonated peptides or proteins are confined in the Penning trap of a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer and exposed to electrons with near-thermal ener-

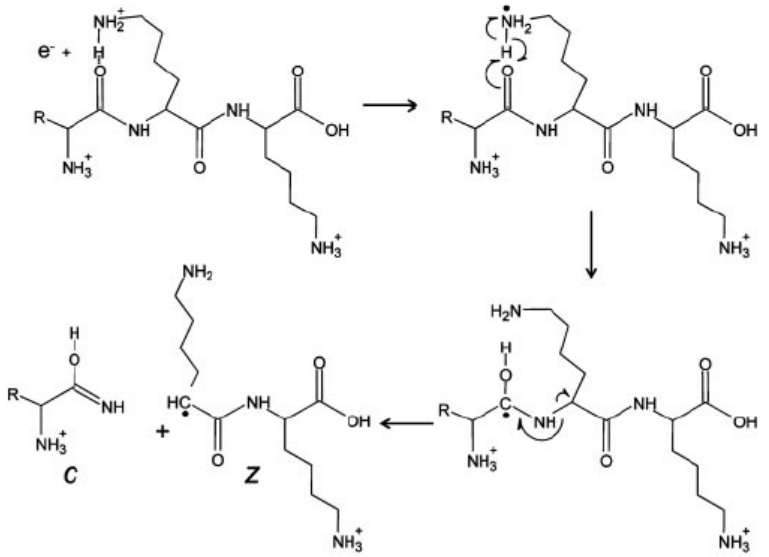
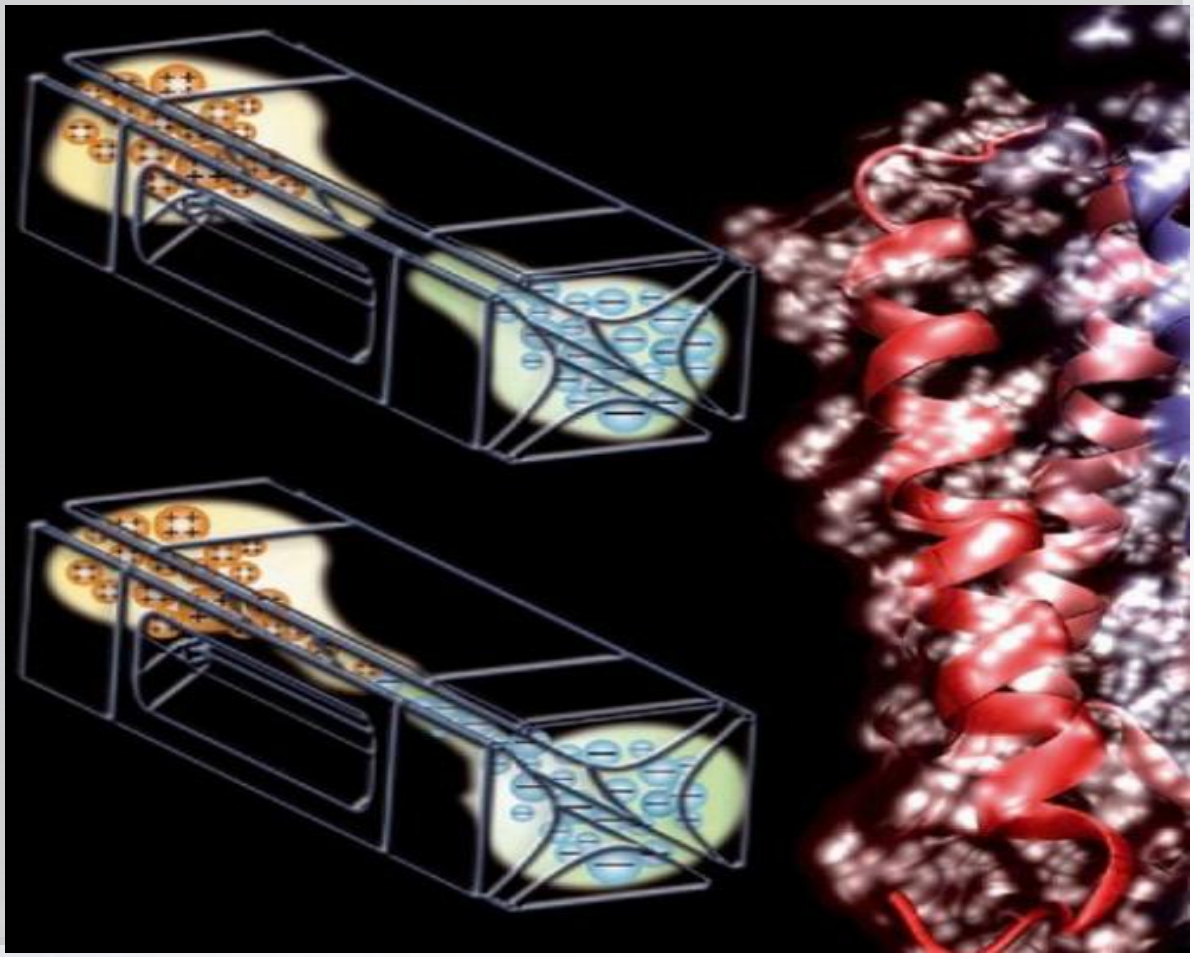


Fig. 1. Fragmentation scheme for production of c- and z-type ions after reaction of a low-energy electron with a multiply protonated peptide.

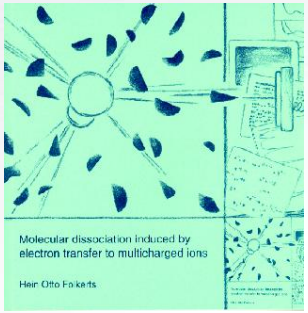
Instrumentations of ETD

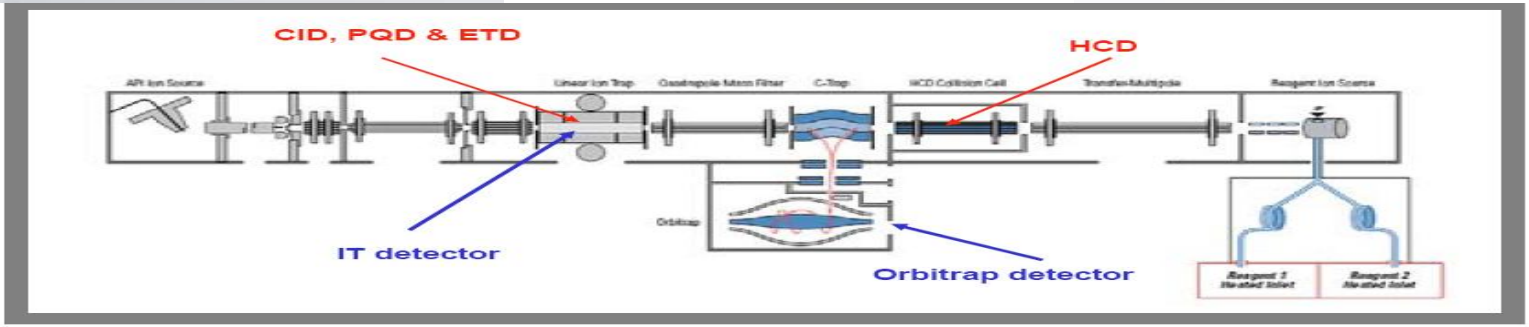
- **Thermo Scientific: LTQ XL 2005**
  LTQ-OT XL 2007
  LTQ-OT Velos 2009
- **Bruker Daltonics: HCTultra 2007**
- **Agilent: 6340 2007**
- **ABI: Q-Trap 2000 2008**
- **Hitachi**

LTQ-ETD (2005)

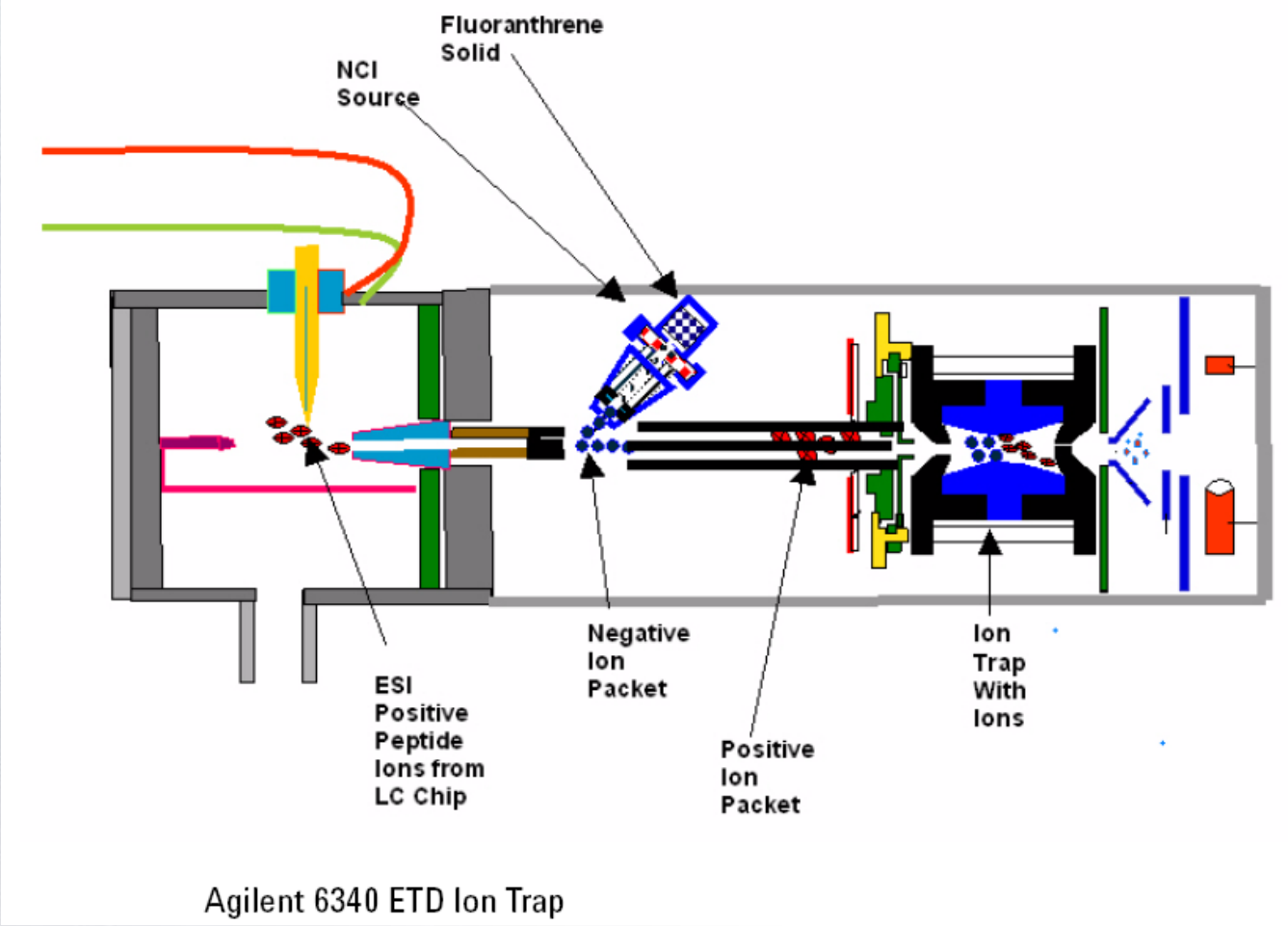


LTQ-OT XL with ETD (2007)



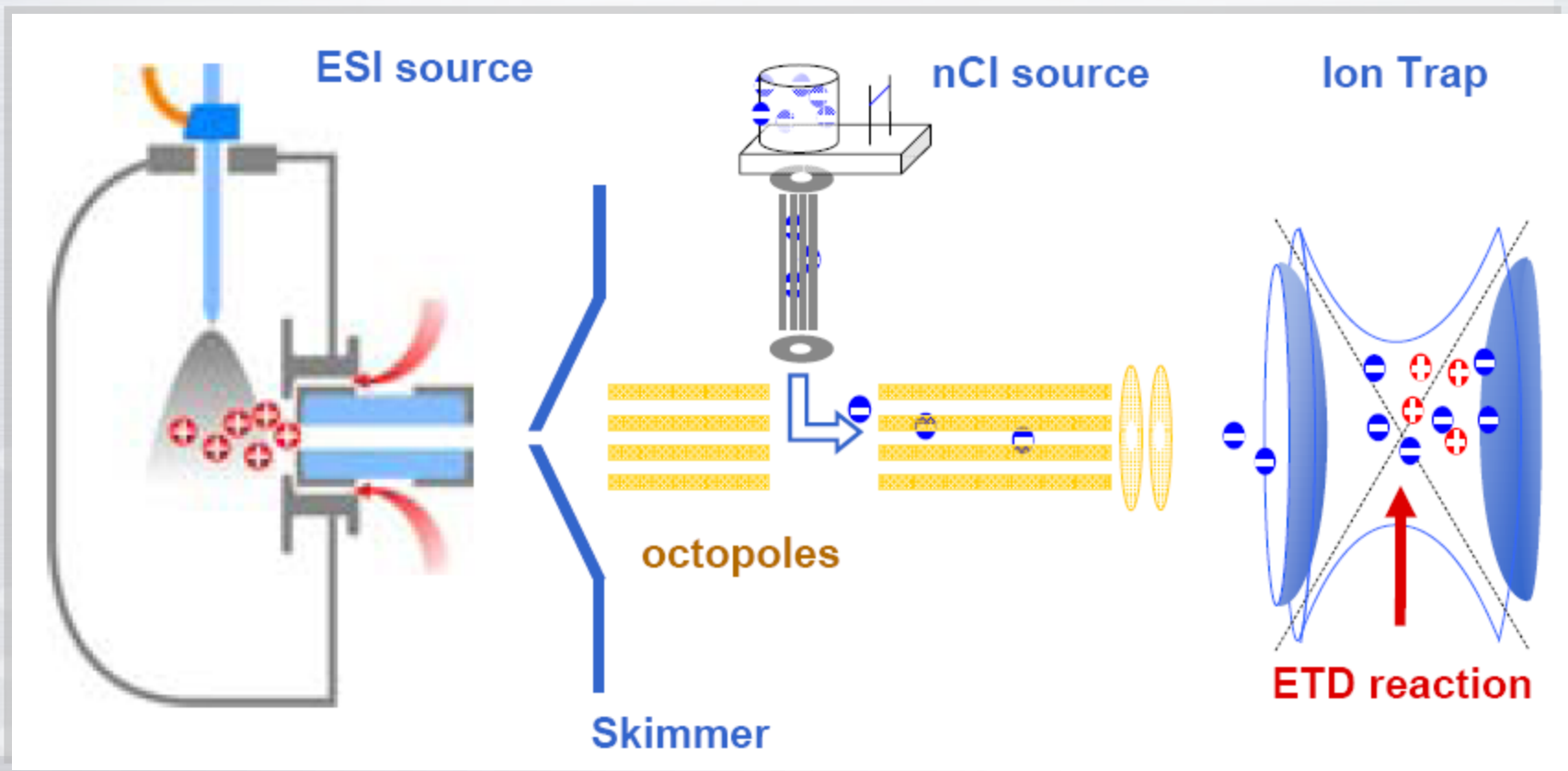


Agilent 6340 ETD



Agilent 6340 ETD Ion Trap

Bruker HCT Ultra PTM Discovery



Others: Shimatsu, Hitachi, …

Data processing of ETD spectra

Software:

(1) Sequest (Bioworks), Proteome Discover

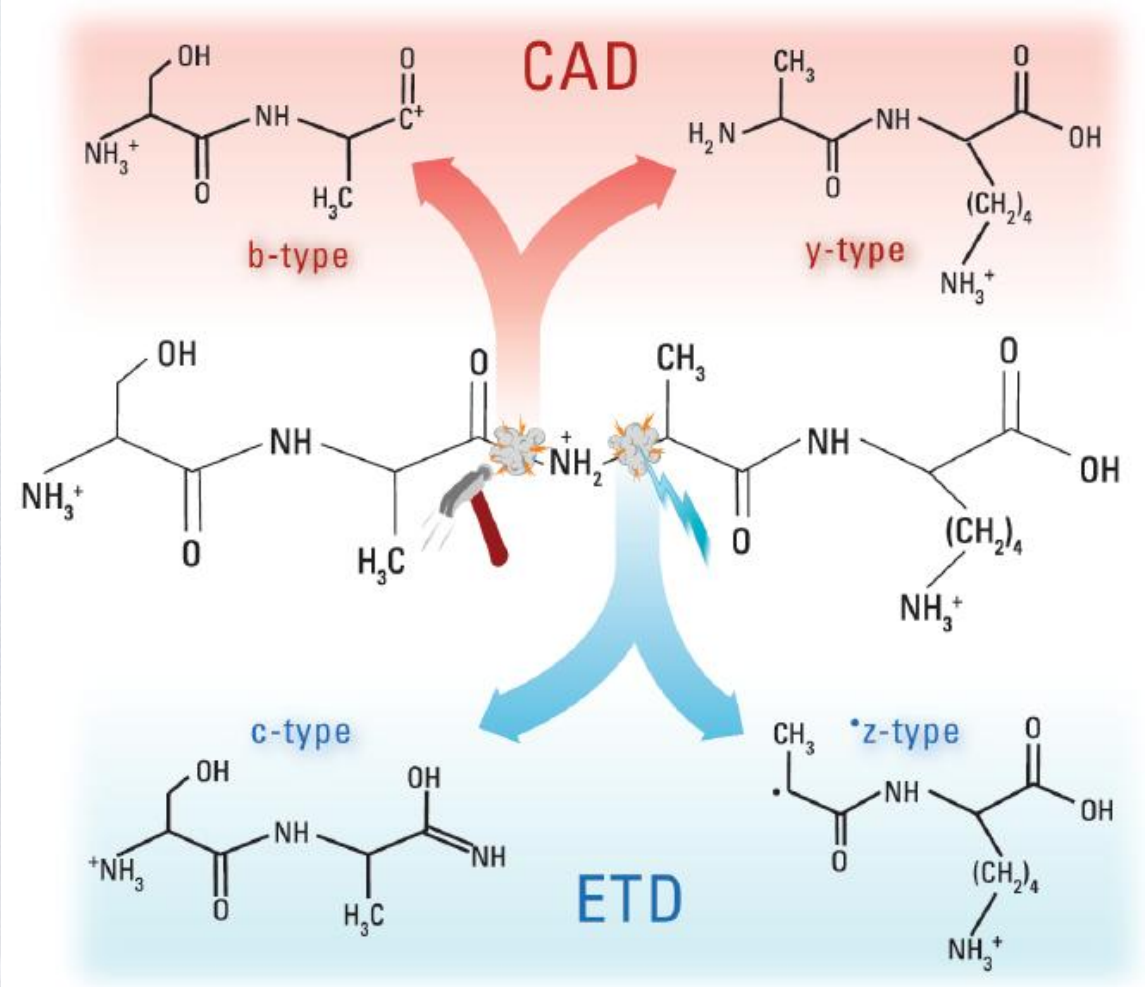
(2) Mascot (ECD/ETD)

(3) OMSSA

(4) X!Tandem

……

Fragmentations: CID and ETD

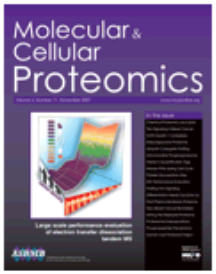


Coon, J. J. (2009) Collisions or electrons? protein sequence analysis in the 21st century. Anal. Chem. 81, 3208–3215

Molecular & Cellular PROTEOMICS

Contents: November 2007, Vol. 6, No. 11

[Index by Author] [Cover Caption]



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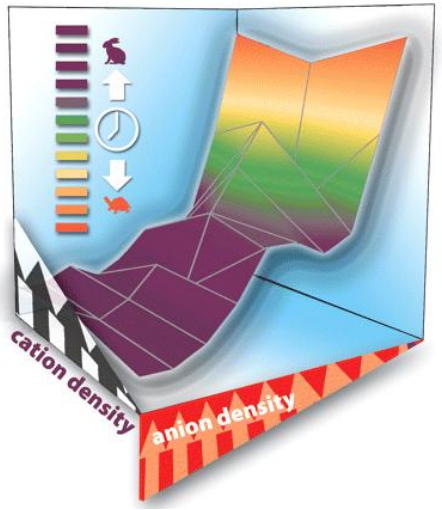

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Clear Get All Checked Abstract(s)

Research:

☐ **Performance Characteristics of Electron Transfer Dissociation Mass Spectrometry**
David M. Good, Matthew Wirtala, Graeme C. McAlister, and Joshua J. Coon
Mol Cell Proteomics 2007 6: 1942-1951. First Published on August 1, 2007; doi:10.1074/mcp.M700073-MCP200
[Abstract] [Full Text] [PDF] Supplemental Data

Complementary of CID and ETD

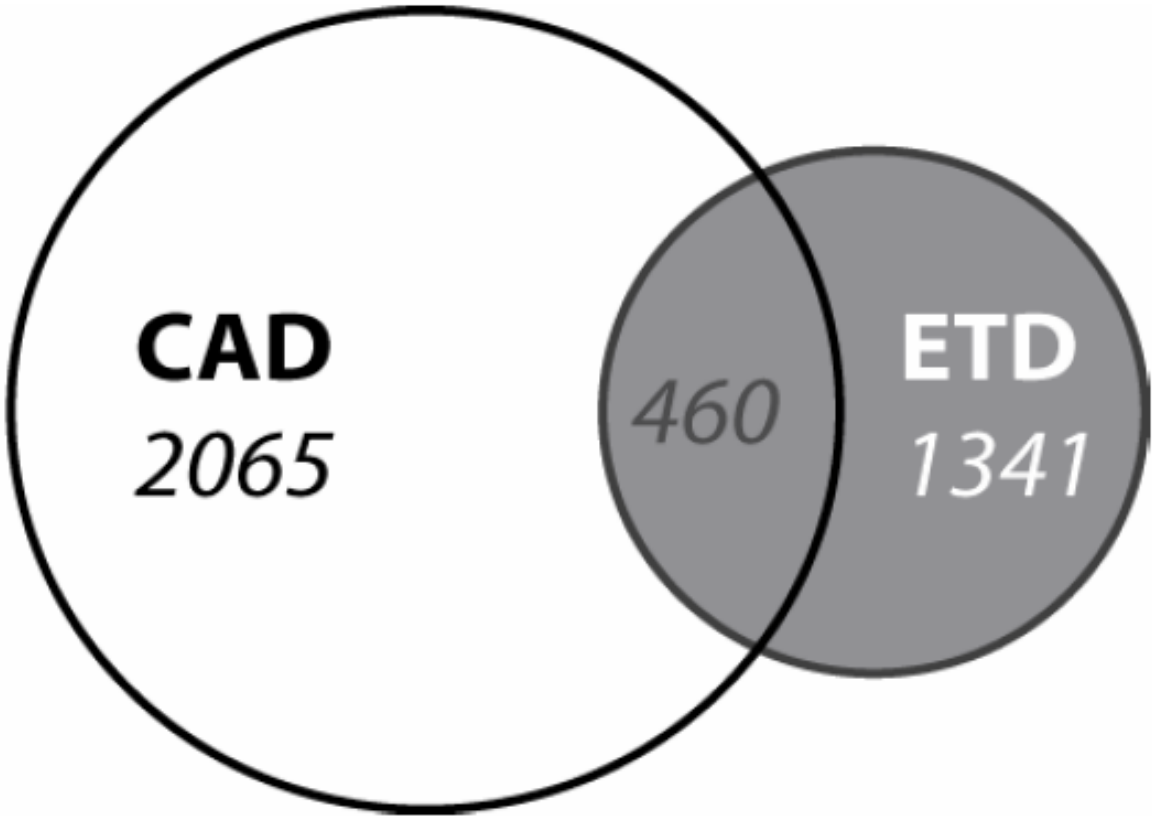
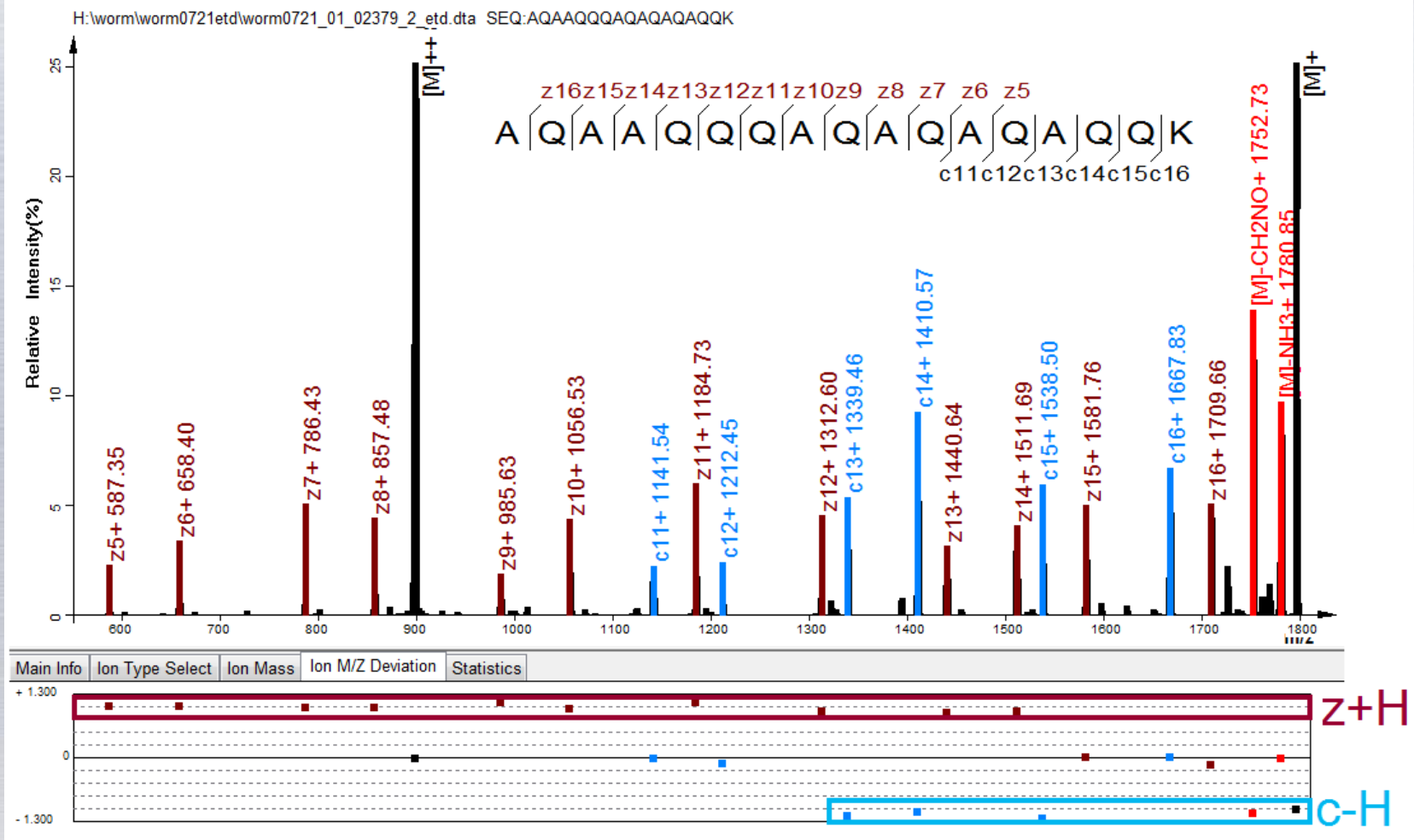


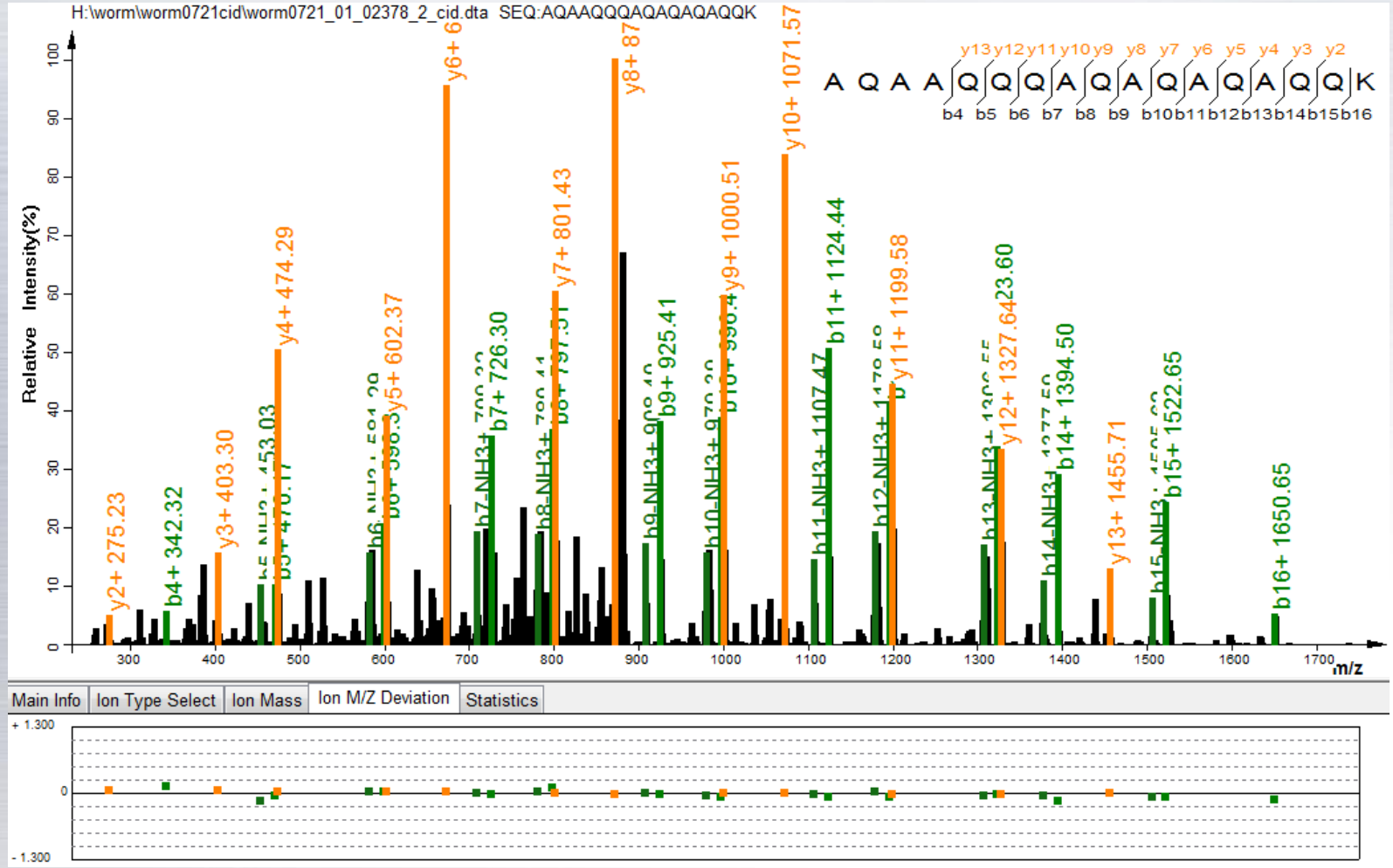
Figure 1. Of the 3866 total peptides sequenced, there was only a ~12% overlap in identifications from ion trap CAD and ETD.

An ETD example



This spectrum was annotated by the software pLabel (http://pfind.ict.ac.cn)

CID partner (the same peptide)



This spectrum was annotated by the software pLabel (http://pfind.ict.ac.cn)

Hydrogen Rearrangement in ECD (+2)

Hydrogen Rearrangement to and from Radical z Fragments in Electron Capture Dissociation of Peptides

Mikhail M. Savitski,* Frank Kjeldsen,* Michael L. Nielsen, and Roman A. Zubarev

Laboratory for Biological and Medical Mass Spectrometry, Uppsala University, Uppsala, Sweden

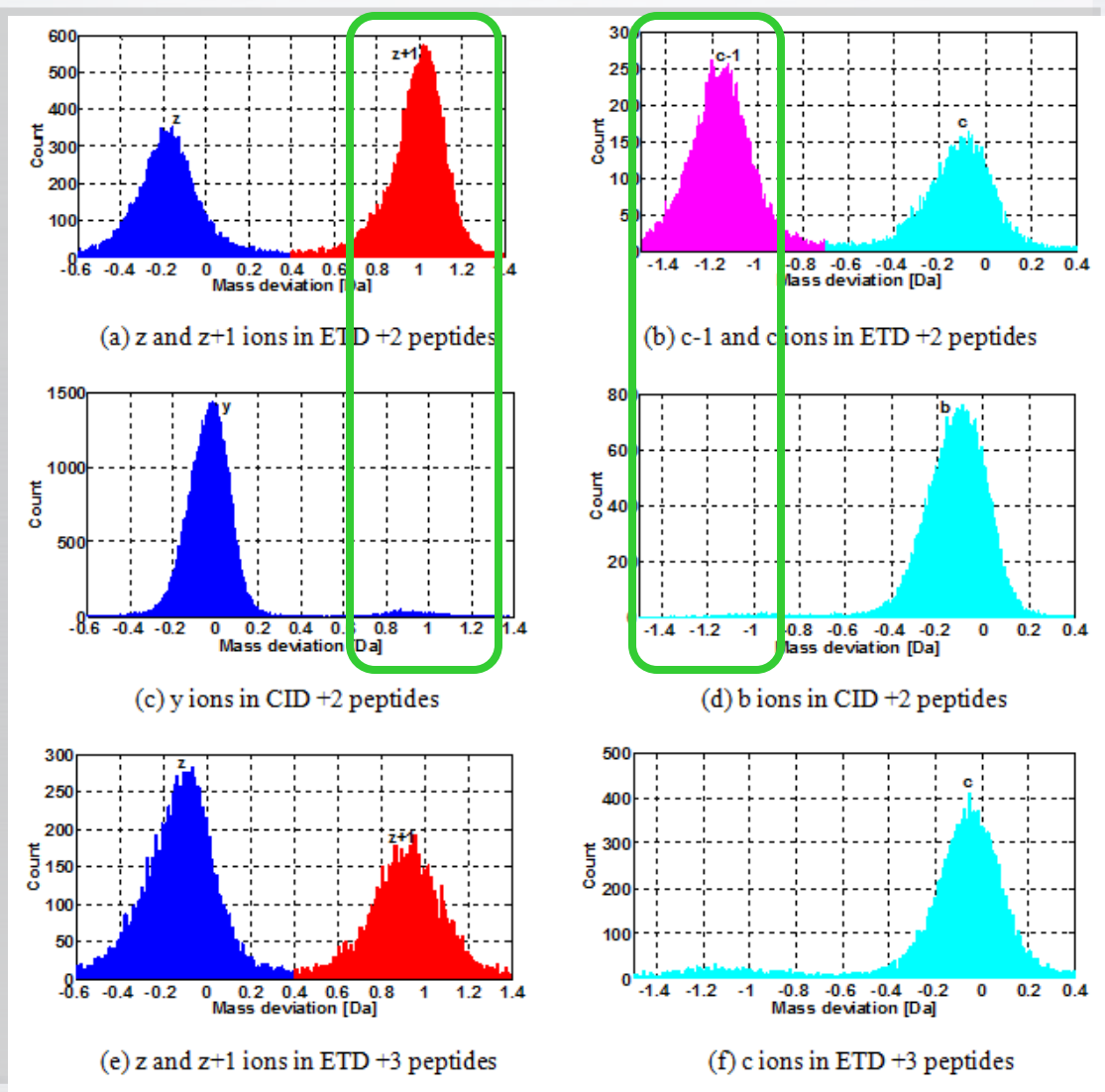
Hydrogen rearrangement is an important process in radical chemistry. A high degree of H· rearrangement to and from z· ionic fragments (combined occurrence frequency 47% compared with that of z·) is confirmed in analysis of 15,000 tandem mass spectra of tryptic peptides obtained with electron capture dissociation (ECD), including previously unreported double H· losses. Consistent with the radical character of H· abstraction, the residue determining the formation rate of $z' = z$· + H· species is found to be the N-terminal residue in z· species. The size of the complementary c_m' fragment turned out to be another important factor, with z' species dominating over z· ions for $m \leq 6$. The H· atom was found to be abstracted from the side chains as well as from α-carbon groups of residues composing the c' species, with Gln and His in the c' fragment promoting H· donation and Asp and Ala opposing it. Ab initio calculations of formation energies of ·A radicals (A is an amino acid) confirmed that the main driving force for H· abstraction by z· is the process exothermicity. No valid correlation was found between the N—C_α bond strength and the frequency of this bond cleavage, indicating that other factors than thermochemistry are responsible for directing the site of ECD cleavage. Understanding hydrogen attachment to and loss from ECD fragments should facilitate automatic interpretation ECD mass spectra in protein identification and characterization, including de novo sequencing. (J Am Soc Mass Spectrom 2007, 18, 113–120) © 2007 American Society for Mass Spectrometry

ETD (ECD) data sets

Table 1. Ten ETD/ECD data sets analyzed

No.	Data set	#Spectra	MS2 Resolution	Instrument	Species	Digestion Enzyme	Precursor Charge States	Reference
1	WORM-A	58,424[a]	Normal [c]	LTQ-Orbitrap with ETD	*C. elegans*	Trypsin	+2, +3	This manuscript
2	WORM-B	60,585[a]						
3	WORM-C	56,525[a]						
4	WORM-H	22,258[a]	High [d]					
5	YEAST-B1	52,520[a]	Normal [c]		*S. cerevisiae*	Lys-C	+2,+3, +4,+5	Ref.36
6	YEAST-B2R1	59,485[a]						
7	YEAST-B2R2	59,007[a]						
8	YEAST-ETcaD	56,019[a]						
9	SwedECD	11,491[b]	High [e]	LTQ-FT with ECD	*Human & E. coli*	Trypsin	+2	Ref.38
10	PhosphorETD	36,617[a]	Normal [c]	LTQ-Orbitrap with ETD	C. *elegans*	Trypsin	+2,+3	This manuscript

Distribution of Fragment mass deviations



+2 ETD
z, c

+2 CID
y, b

+3 ETD
z, c

nature methods
Techniques for life scientists and chemists

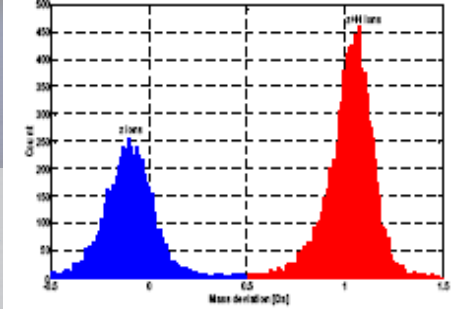
ARTICLE ABSTRACT

Nature Methods **5**, 959 - 964 (2008)
Published online: 19 October 2008 | doi:10.1038/nmeth.1260

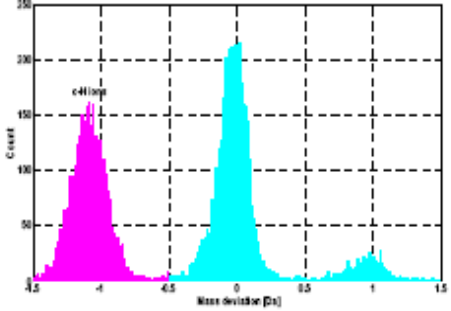
Decision tree–driven tandem mass spectrometry for shotgun proteomics

Danielle L Swaney[1,3], Graeme C McAlister[1,3] & Joshua J Coon[1,2]

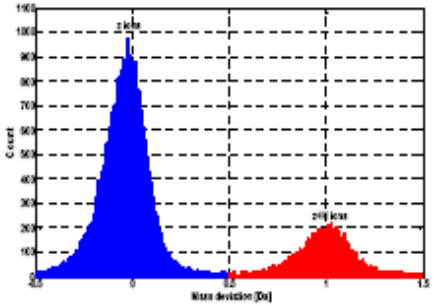
Mass spectrometry has become a key technology for modern large-scale protein sequencing. Tandem mass spectrometry, the process of peptide ion dissociation followed by mass-to-charge ratio (*m/z*) analysis, is the critical component for peptide identification. Recent advances in mass spectrometry now permit two discrete, and complementary, types of peptide ion fragmentation: collision-activated dissociation (CAD) and electron transfer dissociation (ETD) on a single instrument. To exploit this complementarity and increase sequencing success rates, we designed and embedded a data-dependent decision tree algorithm (DT) to make unsupervised, real-time decisions of which fragmentation method to use based on precursor charge and *m/z*. Applying the DT to large-scale proteome analyses of *Saccharomyces cerevisiae* and human embryonic stem cells, we identified 53,055 peptides in total, which was greater than by using CAD (38,293) or ETD (39,507) alone. In addition, the DT method also identified 7,422 phosphopeptides, compared to either 2,801 (CAD) or 5,874 (ETD) phosphopeptides.



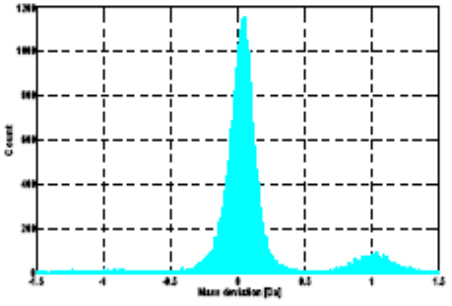
(a) z and z+H ions (+2 peptides)



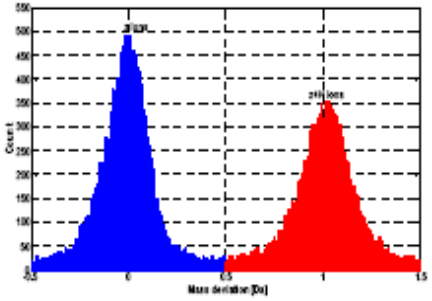
(b) c-H and c ions (+2 peptides)



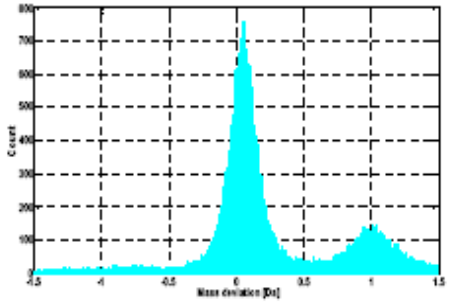
(c) z and z+H ions (+3 peptides)



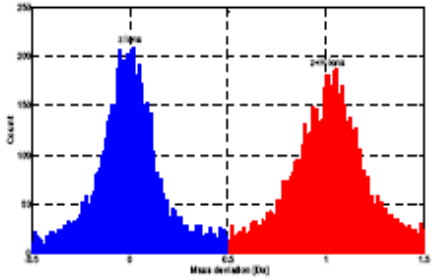
(d) c ions (+3 peptides)



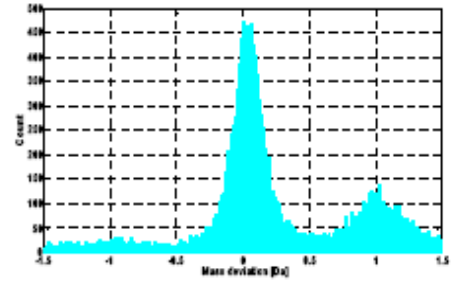
(e) z and z+H ions (+4 peptides)



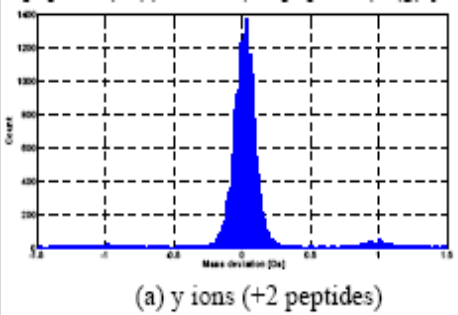
(f) c ions (+4 peptides)



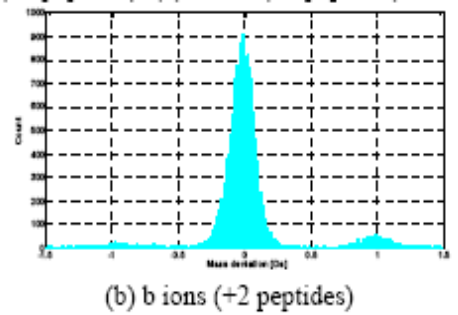
(g) z and z+H ions (+5 peptides)



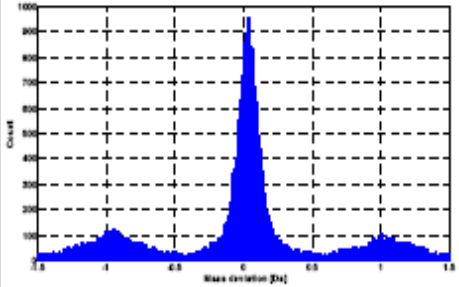
(h) c ions (+5 peptides)



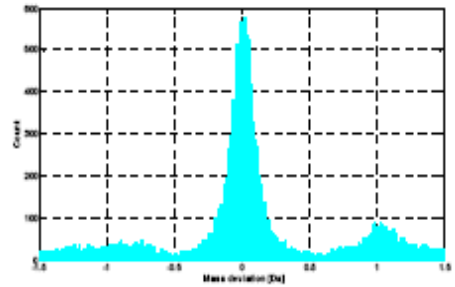
(a) y ions (+2 peptides)



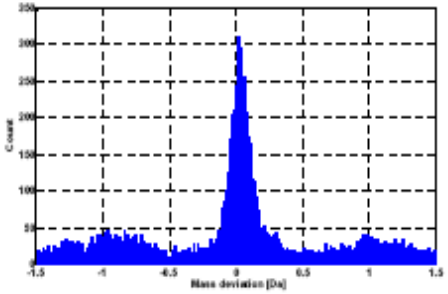
(b) b ions (+2 peptides)



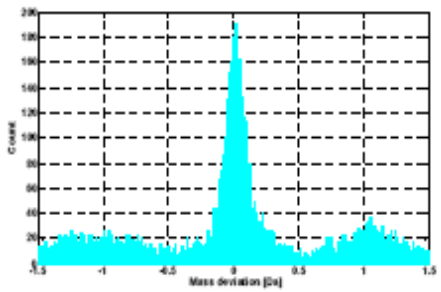
(c) y ions (+3 peptides)



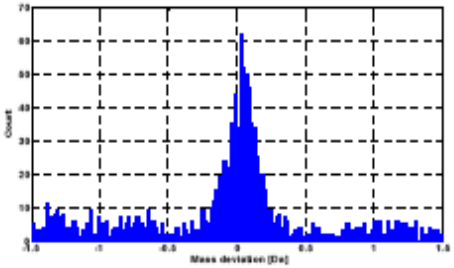
(d) b ions (+3 peptides)



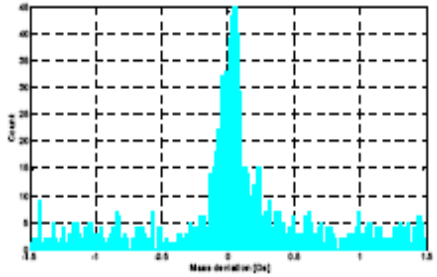
(e) y ions (+4 peptides)



(f) b ions (+4 peptides)

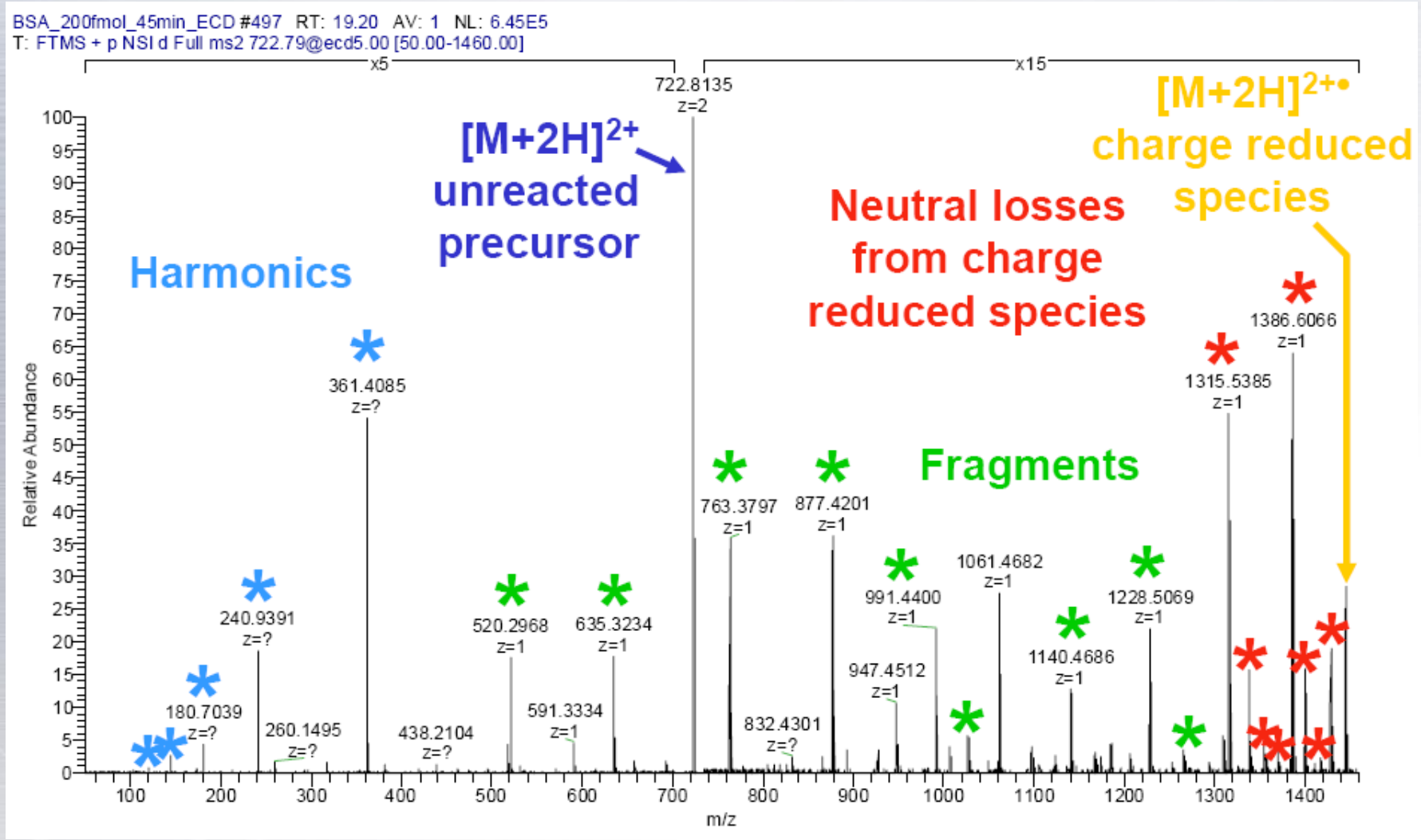


(g) y ions (+5 peptides)

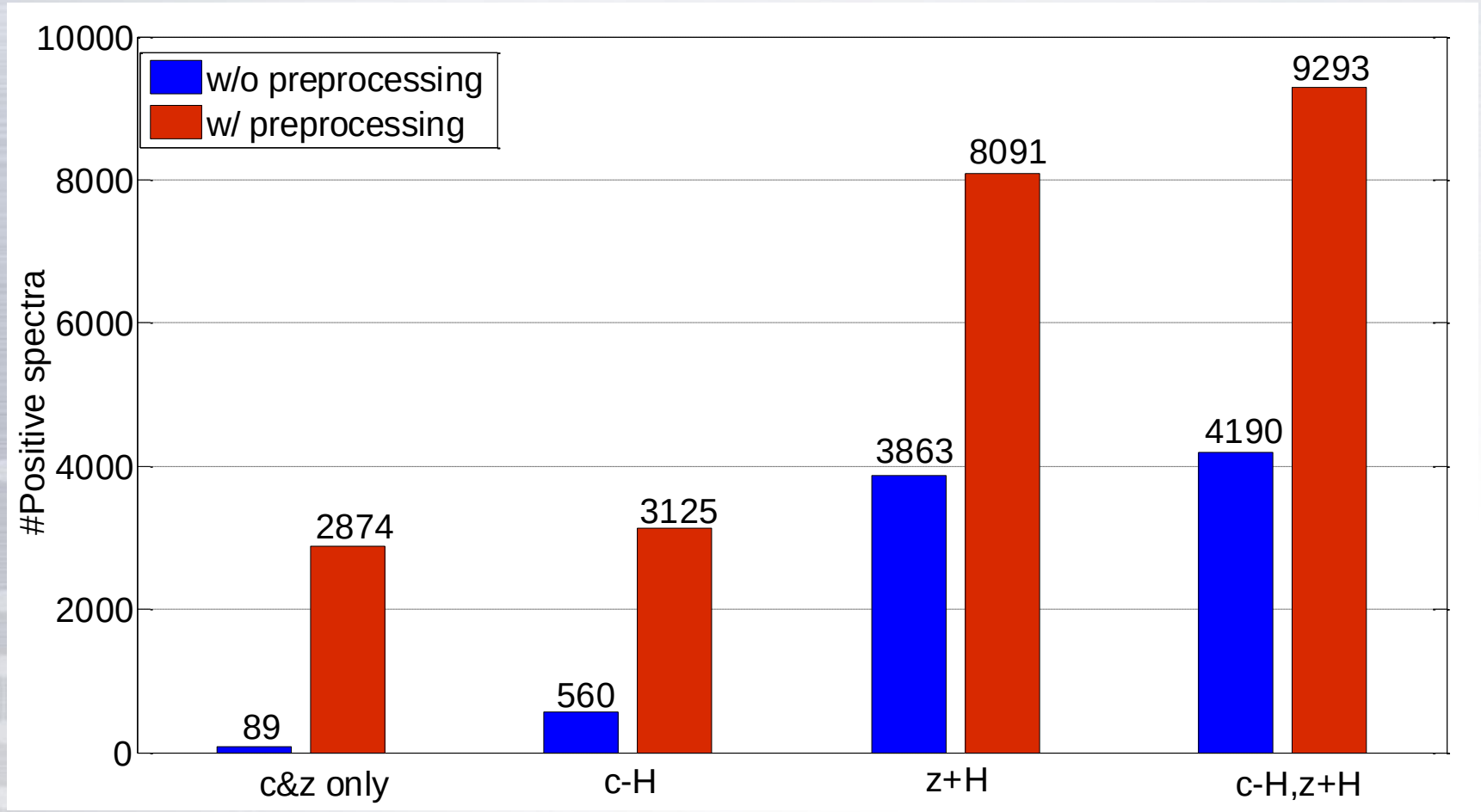


(h) b ions (+5 peptides)

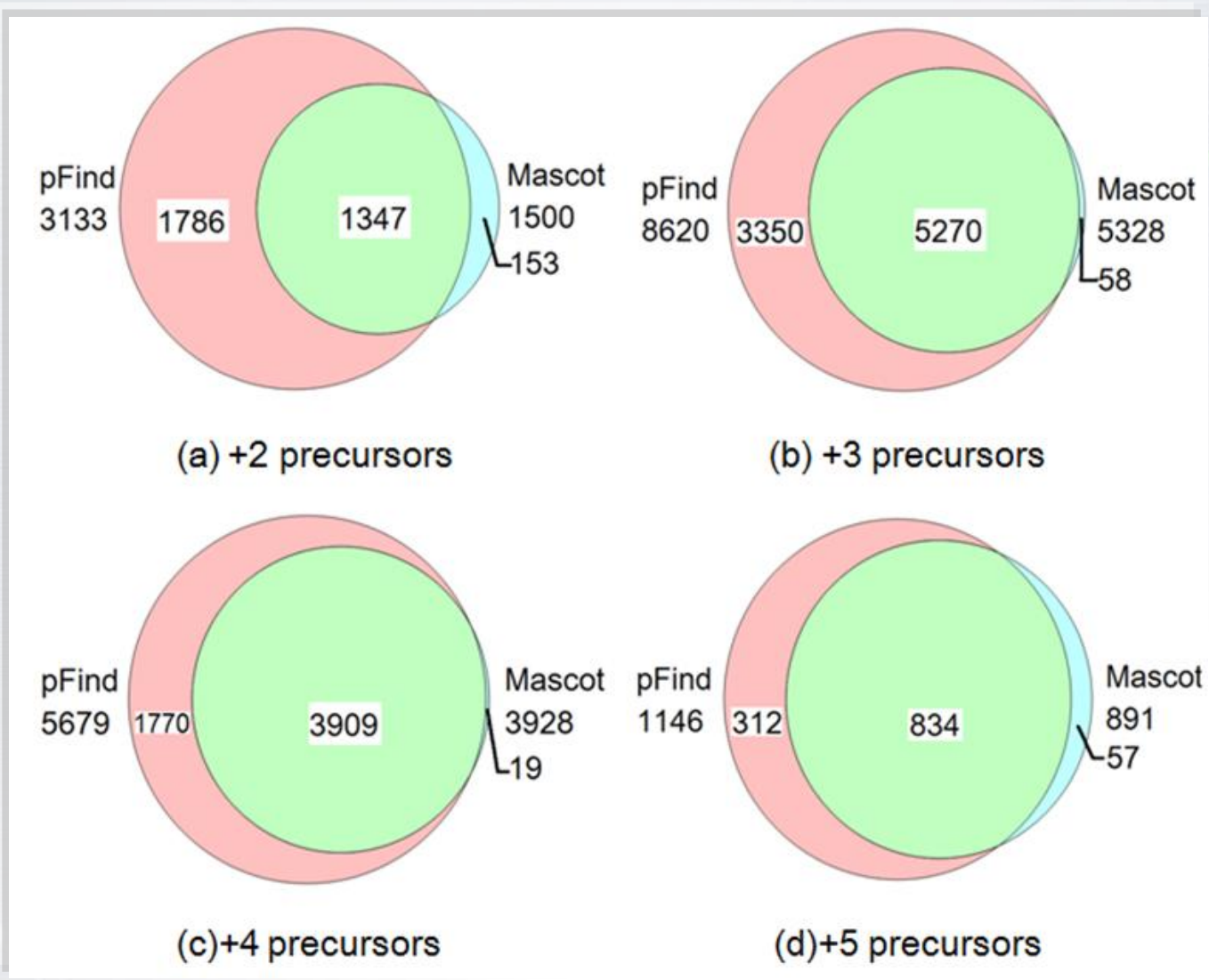
An ECD spectrum



Test experiments on pFind 2.1



Overlapping between pFind vs. Mascot



Phosphopeptide ID by pFind and Mascot

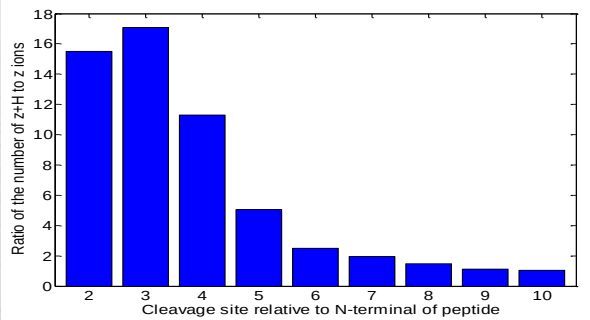
Table 6. Comparison of pFind and Mascot on Phosphopeptide ETD data

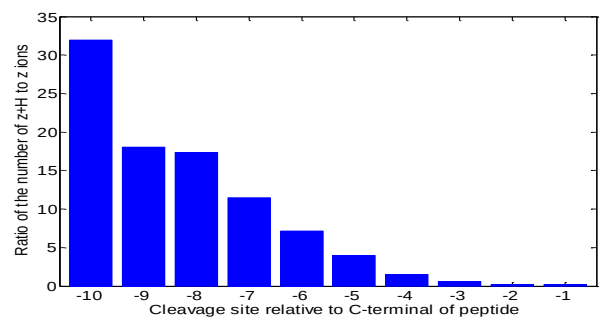
	Identifications (FDR=1%)	Mascot	pFind	Mascot∩pFind[a]	Mascot∪pFind[b]
Phosphopeptides	#spectra	596	1581	560	1622
	#peptides	351	612	329	637
	#proteins	516	792	481	827

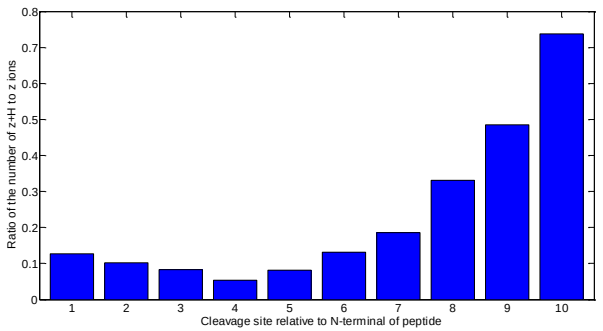
a: Number of overlapping results between Mascot and pFind.

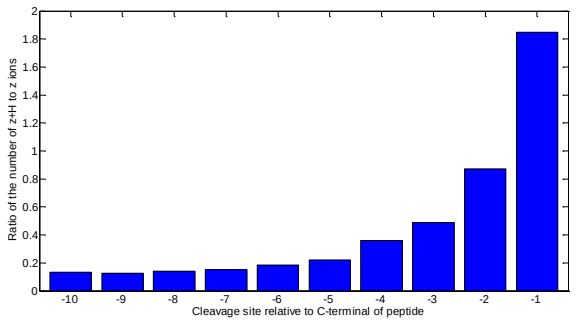
b: Number of combined results of Mascot with pFind.

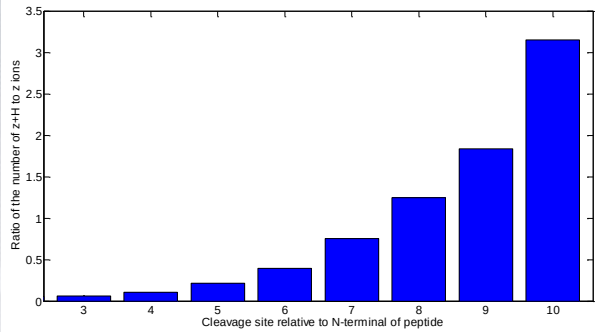
Hydrogen Rearrangement Loc.

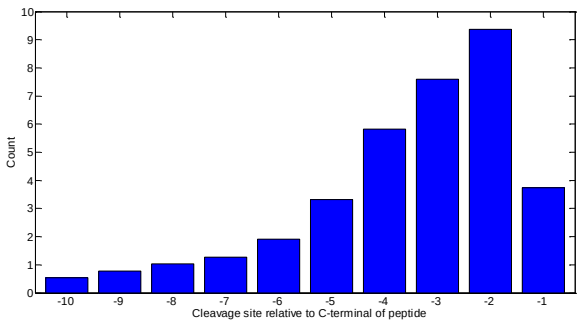




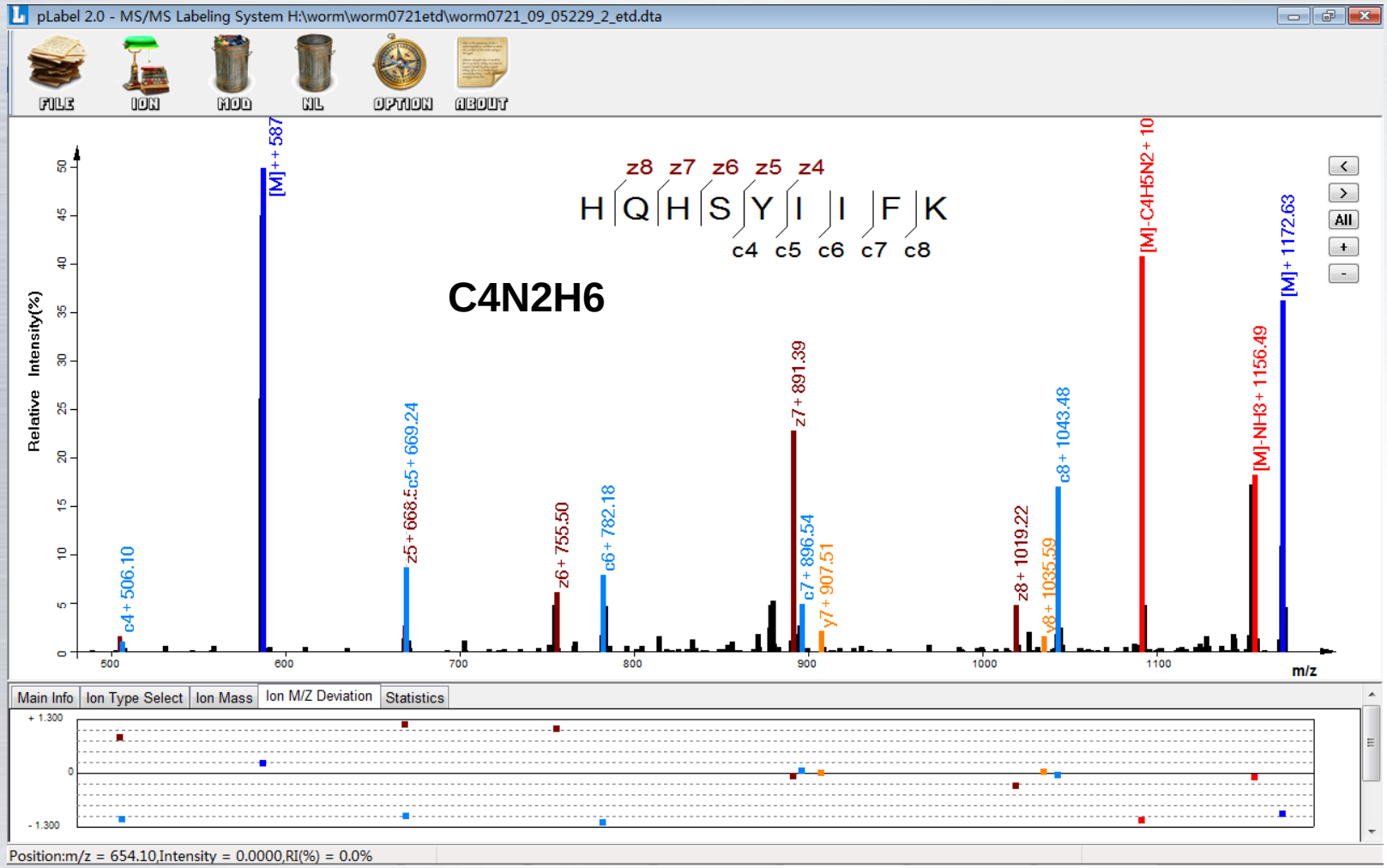




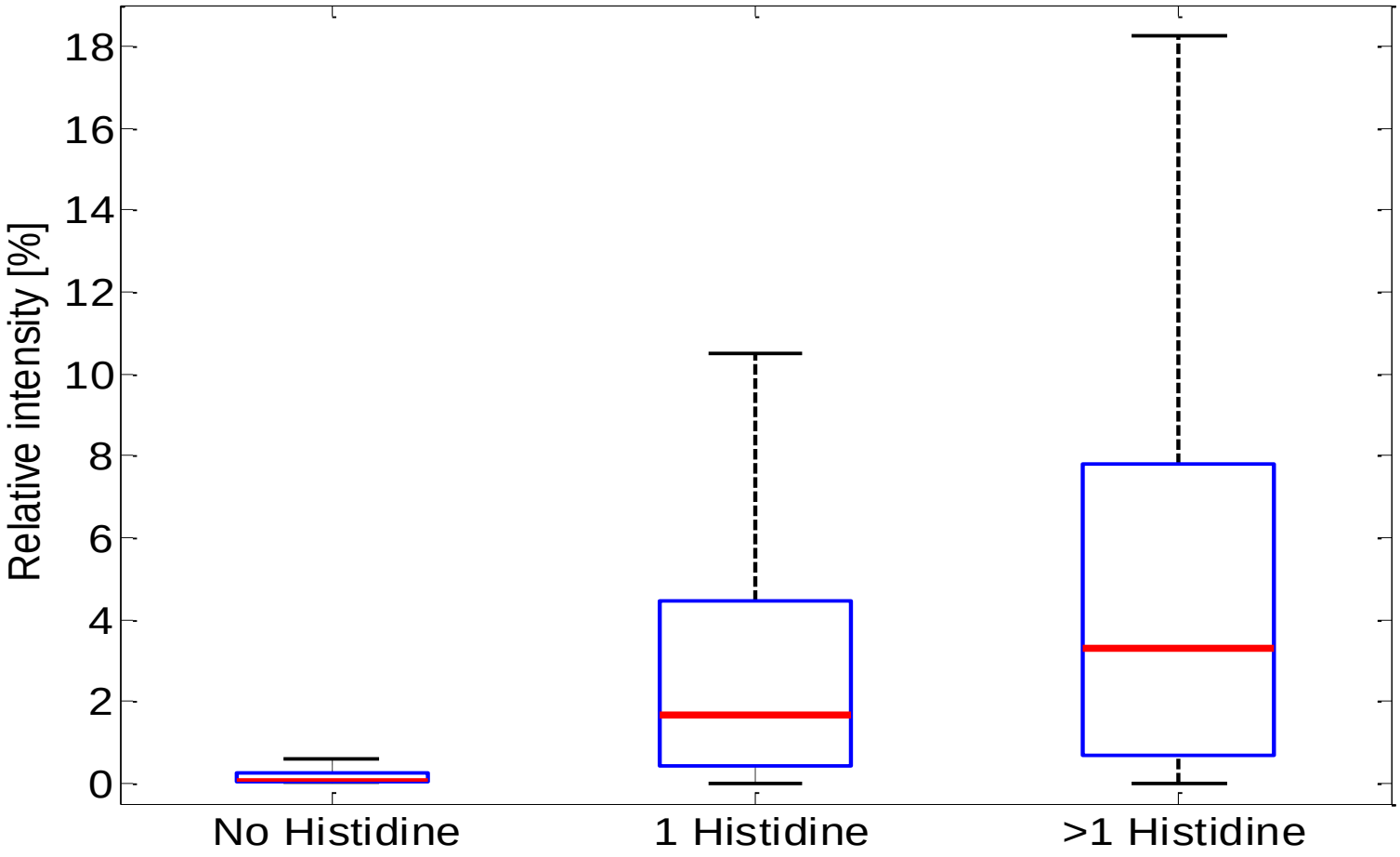




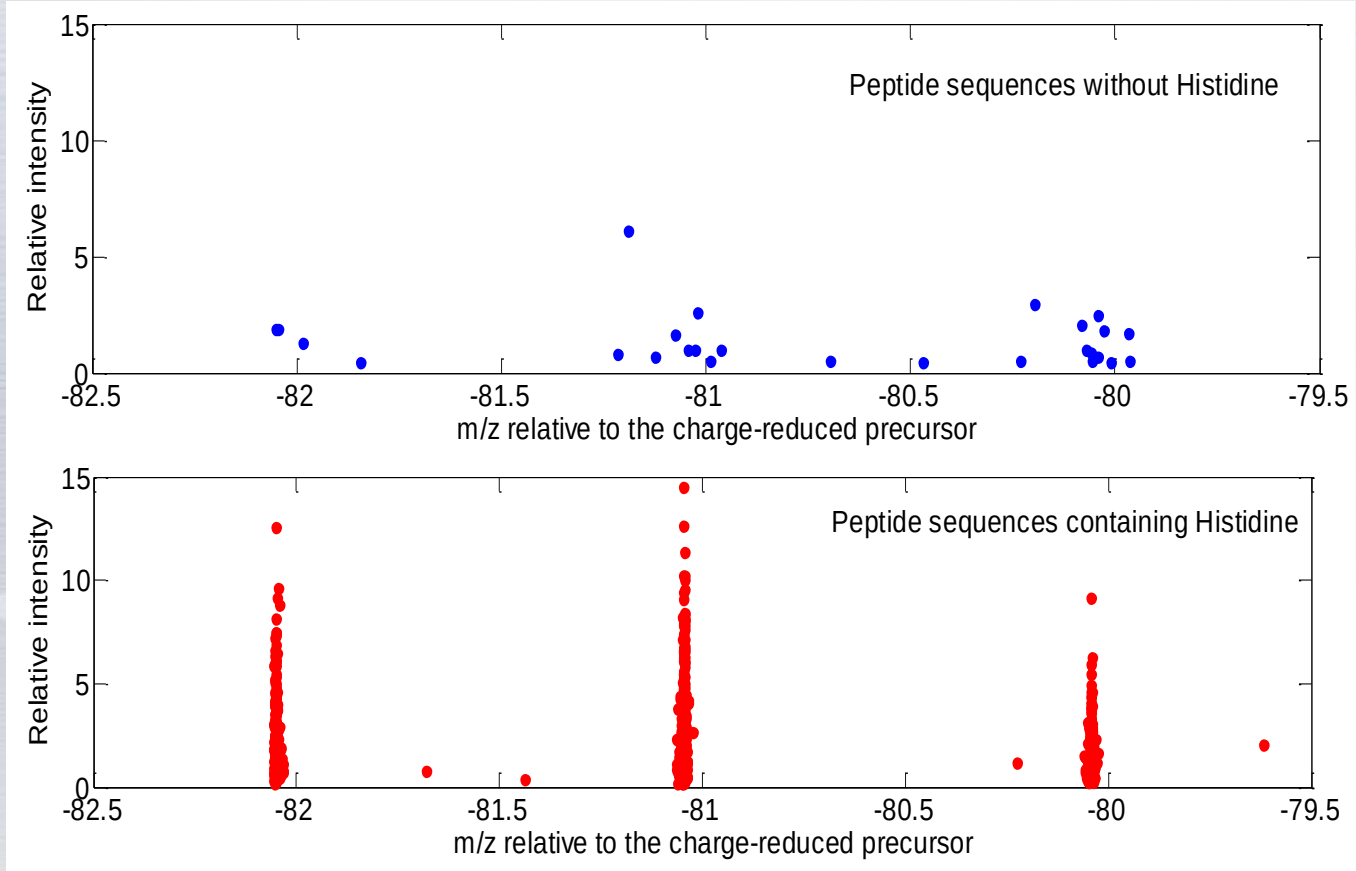
Side Chain Loss



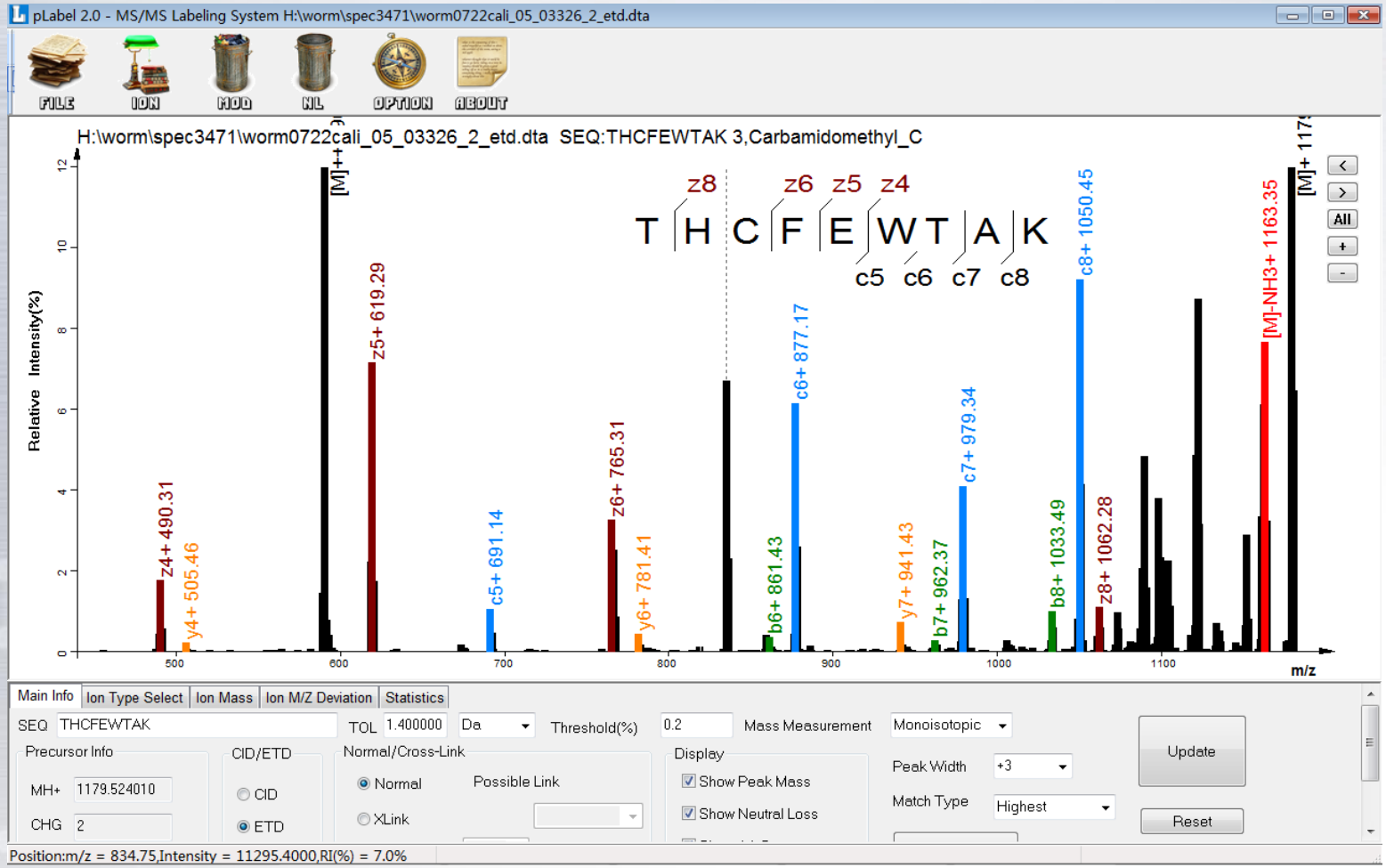
Histidine's side chain losses



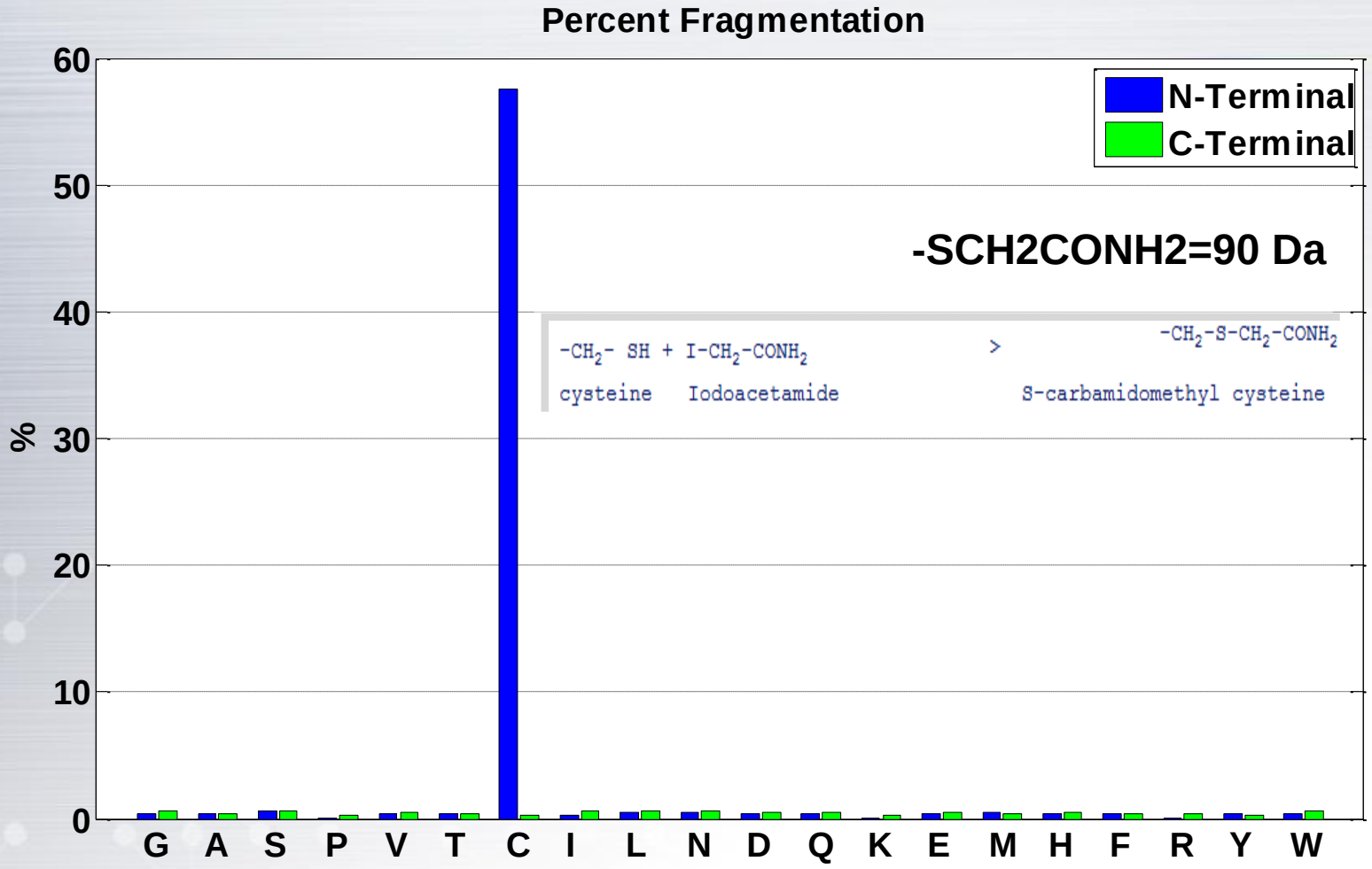
Side Chain Loss



Side Chain Loss C-90



Side Chain Loss C-90 (SwedECD,11491)



Summary on ETD Fragmentation patterns

Table 2. Characterization on fragmentation patterns of ECD and ETD

Items	ECD	Reference	ETD	Reference
Backbone fragmentation	Main ion types are the radical z˙ and c.	[11]	Main ion types are the radical z˙ and c with minor a˙ and y ions.	[12]
Proline N-terminal cleavage	Suppressed due to the double bond-linking of Proline.	[11]	Suppressed due to the double bond-linking of Proline.	[12]
Charge-reduced and neutral loss species	Very typical, often with the relative higher intensity than fragment ions.	[49, 50]	Very typical, often with the relative higher intensity than fragment ions. ETcaD or SA can reduce the charge-reduced species, increasing z or c ions yield.	Detailed analysis not reported
Side chain loss of peptide containing Histidine	A prominent peak for ~81 Da loss from the intact peptide(side chain loss of Histidine: $C_4N_2H_6$)	[51]	A prominent peak for ~81 Da loss from the intact peptide(side chain loss of Histidine: $C_4N_2H_6$) with a very high specificity.	This manuscript
Side chain loss of fragment ions	w and u ions in Hot-ECD (HECD)	[51]	Not typical	This manuscript
Side chain loss of fragment ions containing Carbamido-methylated Cysteine	A prominent peak for ~90 Da loss from z ions(side chain loss of Carbamidomethylated Cysteine SCH_2CONH_2)	[46]	A prominent peak for ~90 Da loss from z ions with its N-terminal Carbamidomethylated Cysteine (side chain loss of SCH_2CONH_2).	This manuscript
Harmonic peaks	Peaks at 1/2, 1/3, … even till to 1/6 of the precursor's m/z	[52]	Not observed	[52]
HR on +2 peptides	First HR report on two model peptides; Statistics of HR on z ions based on a large-scale ECD spectra. There was a 47% occurrence frequency for HR z ions.	[37]; [38]	(1) SA can increase the yield of z and c ions with the propensity to produce more HR ions; (2) A higher proportion of c-H in ETD than in ECD (Figures S6b and S6d). Both z+H and c-H ions cannot be ignored; (3) A larger z ion has a lower propensity to abstract a hydrogen, and vice versa.	[35]; This manuscript
HR on >=+3 peptides	Not reported	Not reported	(1) z+H is still obviously observed although its occurrence frequency decreases when compared with +2 peptides; (2) c-H is not typical and can be omitted; (3) A larger z ion has a higher possibility to abstract a hydrogen, opposite to that of +2 peptides.	This manuscript

JPR online publication, 2010-09-30

research articles Journal of proteome research

Improved Peptide Identification for Proteomic Analysis Based on Comprehensive Characterization of Electron Transfer Dissociation Spectra

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Received June 25, 2010

In recent years, electron transfer dissociation (ETD) has enjoyed widespread applications from sequencing of peptides with or without post-translational modifications to top-down analysis of intact proteins. However, peptide identification rates from ETD spectra compare poorly with those from collision induced dissociation (CID) spectra, especially for doubly charged precursors. This is in part due to an insufficient understanding of the characteristics of ETD and consequently a failure of database

<生物化学与生物物理进展>

PIBB 生物化学与生物物理进展
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2010, 37(1): 94~102
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基于电子捕获裂解/电子转运裂解串联质谱技术的蛋白质组学研究 *

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摘要 蛋白质组学的兴起带动了质谱技术的快速发展，而质谱技术的进步则拓宽了蛋白质组学研究问题的广度. 最近 10 年内，肽段或完整蛋白质在质谱仪中的裂解技术——电子捕获裂解(electron capture dissociation, ECD)与电子转运裂解(electron transfer dissociation, ETD)逐渐发展起来. ECD 和 ETD 在蛋白质组学中的应用，特别是在蛋白质的翻译后修饰鉴定和“自顶而下(Top-down)”的完整蛋白质裂解研究中已经展示出了诱人的前景. 对 ECD 和 ETD 的基本原理、质谱特点、仪器实现、数据解析算法与软件开发，以及在蛋白质组学中的应用进展等方面进行了比较系统全面的阐述，并对当前的研究问题、面临的技术挑战与未来的发展趋势等方面作了深入剖析.

关键词 电子捕获裂解，电子转运裂解，碰撞诱导裂解，串联质谱技术，计算蛋白质组学
学科分类号 Q51，TP39 DOI: 10.3724/SP.J.1206.2009.00352

Informatics on ETD data processing-Coon

- **“As we move forward, I predict newer search engines built around ETD fragmentation patterns, rather than adapted from a CAD point of view, will further improve ETD performance.”**
- **ETcaD: H atom loss or gain c-type ions can lose a H atom to become c•-type, while z•-type fragments gain the H atom to generate z-type ions.**
- **“However, the database search algorithms we used (OMSSA) had difficulty with the 1-Da ambiguity of many newly generated fragment ions. I predict that with further development this search algorithm issue will likely be resolved, and we can expect the ETcaD method to offer excellent performance across a very broad precursor *m/z* range.”**

Uppsala Conference on ECD & ETD

- 1st 2003, between Stockholm and Helsinki
- 2nd 2004, Edinburg,UK
- 3rd 2005, Seattle,USA *(ETD)*
- 4th 2006, Hong Kong,China
- 5th 2007, Paris,France
- 6th 2008, Madison,USA
- 7th 2009, Nara,Japan
- 8th 2011, Villars-sur-Ollon, Swiss
  Feb. 6-10

6th Uppsala Conference on ECD & ETD

Topics (2008):

(1) ion-electron interaction fundamentals

(2) instrumentation development

(3) applications: proteomics and PTM analysis

(4) relevant bioinformatics (Informatics)

(2005)

ABRF-iPRG 2011

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Research • Technology
Communication • Education

iPRG-2011: Proteome Informatics Research Group Study:
Identification of Electron Transfer Dissociation (ETD) Mass Spectra

Dear Fellow ABRF Member,

The field of mass spectrometry based proteomics has seen several key innovations over the last several years, including novel experimental methods, new instruments, and unique fragmentation strategies. The latter, in the form of electron capture dissociation (ECD) and the more widely applicable electron transfer dissociation (ETD) have captured the imaginations of many researchers, expanding their ability to identify and analyze peptides and proteins. However, since ECD/ETD spectra differ substantial from more traditional collision induced dissociation (CID) spectra in both their prominent ion series as well as their preferred bond-breaking characteristics, the (automatic) interpretation of ECD/ETD spectra requires novel algorithm optimizations. Efficient identification of ECD/ETD spectra thus remains an active and exciting field of proteomics informatics research.

Acknowlegement

Meng-Qiu Dong's Lab, NIBS

pFind Group