ECD, ETD, PTR and IRMPD ionizace a jejich použití v proteomice

Petr Verner, Thermo Scientific, Praha

- Principles of IRMPD, ECD, ETD, and PTR and
- Hardware
- Applications to Proteomics

- Activation methods prior to ECD
- AI-ECD - (Activation by collision with background gas)
- “Plasma”-ECD (Activation by collision with background gas and concurrent introduction of electrons and analyte ions)
- ICR cell heating (Activation by heating the ICR cell from ambient room temperature up to 175°C)
- IRMPD Activation by absorption of photons

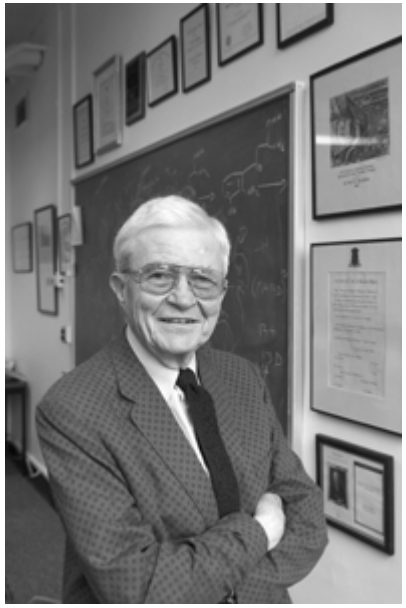
Mild activation by IRMPD prior to ECD breaks weak non-convalent bonds and greatly enhances the number of identified fragment ions.

Advantage of IRMPD over CID:
not dependent on size (MW) of the protein

- ECD

ECD + IRMPD

ProSight PC search results of 7+ charged molecular ion of ubiquitin



Introduced in 1998 by Fred McLafferty, Zubarev and Kelleher

J.Am.Chem.Soc.120 (1998) 3265-3266

Requires high vacuum

Electron capture dissociation typically involves a multiply protonated molecule *M* interacting with a free electron to form an odd-electron ion

$$[M + nH]^{n+} + e^- \rightarrow \left[[M + nH]^{(n-1)+}\right]^* \rightarrow fragments$$

Liberation of the electric potential energy (up to 6eV) results in fragmentation of the product ion.

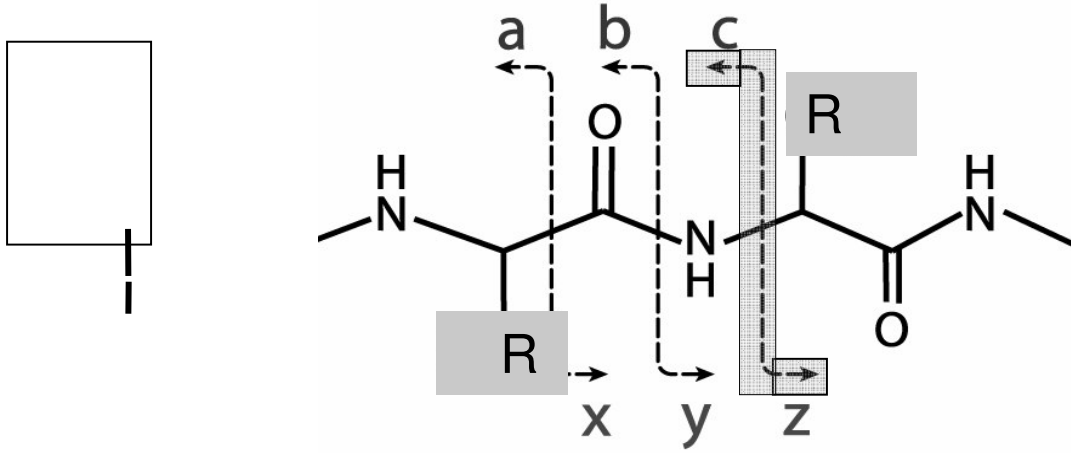
Peptide and Protein Sequence Analysis by Electron Transfer Dissociation (ETD) Mass Spectrometry

Proc. Natl. Acad. Sci. USA, ***2004****, 101, 9528-9533*

Authors: Syka, JEP; Coon, JJ; Schroeder, MJ; Shabanowitz, J; and Hunt, DF

$$[M + 3H]^{3+} + e^- \rightarrow [M + 3H]^{2+\bullet}$$

$$[M + 3H]^{2+\bullet} \rightarrow \underbrace{[C+2H]^{1+}}_{C} + \underbrace{[Z+H]^{1+\bullet}}_{Z}$$



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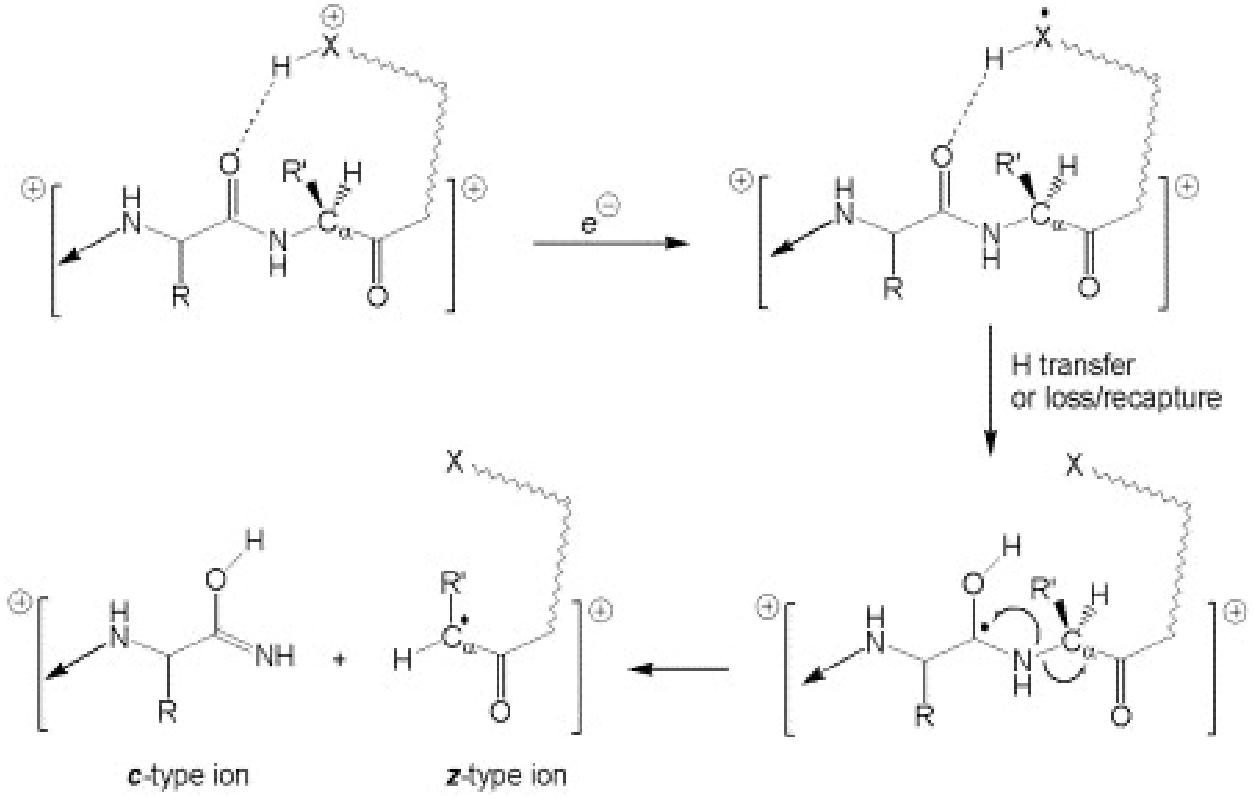
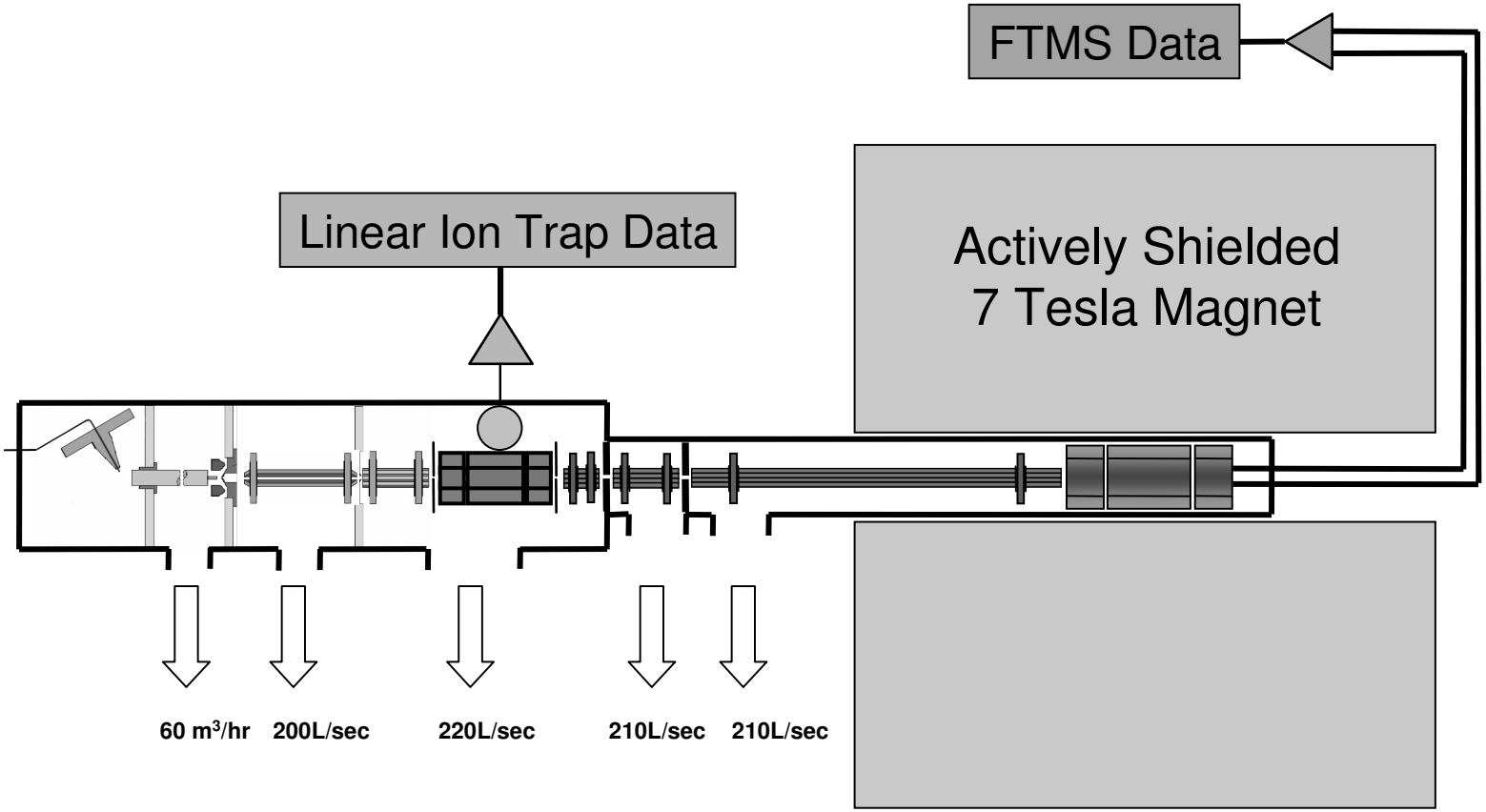


- Use of ECD
  - Phosphopeptides
  - Other labile PTM
  - Highly Basic Peptides
  - Long Peptides
  - When you want more information than CID can provide



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Requires high vacuum

LTQ FT Ultra

- Ultra high res., Accurate Mass
- Top Down, Biomarkers, PTMs

and PEAKS *de novo*

ThermoFisher SCIENTIFIC

- **Ion/Ion Reaction Chemistry**
  - **ETD v.s. PTR**
  - **ETD Fragmentation**
- When Should I Use ETD
  - Phosphopeptides
  - Other labile PTM
  - Highly Basic Peptides
  - Long Peptides
  - When you want more information than CID can provide

Types of Ion/Ion Reactions

Radical Anion

Electron Transfer

$(M + 5H)^{5+} + A^{-\bullet} \rightarrow (M + 5H)^{4+\bullet} + A$

Proton Transfer

$(M + 5H)^{5+} + A^{-} \rightarrow (M + 4H)^{4+} + HA$

Anion Attachment

$(M + 5H)^{5+} + A^{-} \rightarrow (M + 4H + Y)^{4+}$

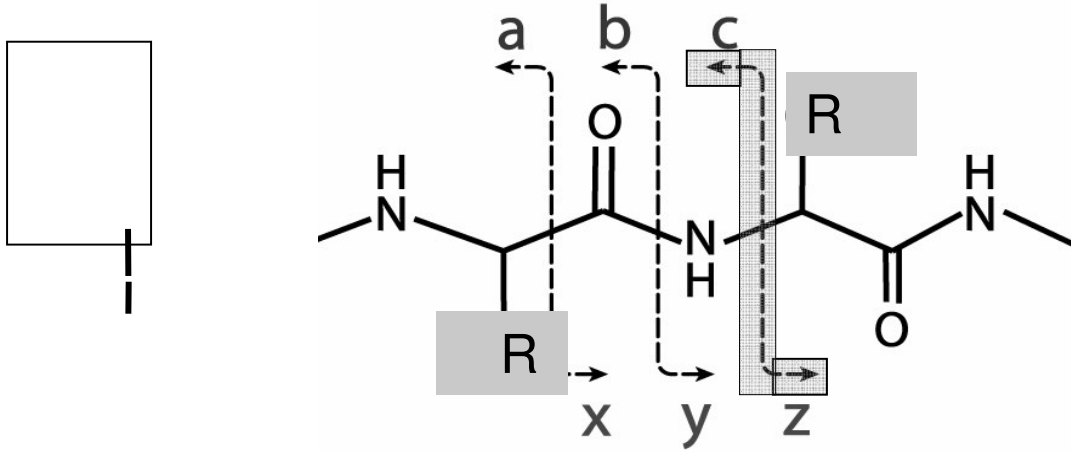
Peptide and Protein Sequence Analysis by Electron Transfer Dissociation (ETD) Mass Spectrometry

*Proc. Natl. Acad. Sci. USA, **2004**, 101, 9528-9533*

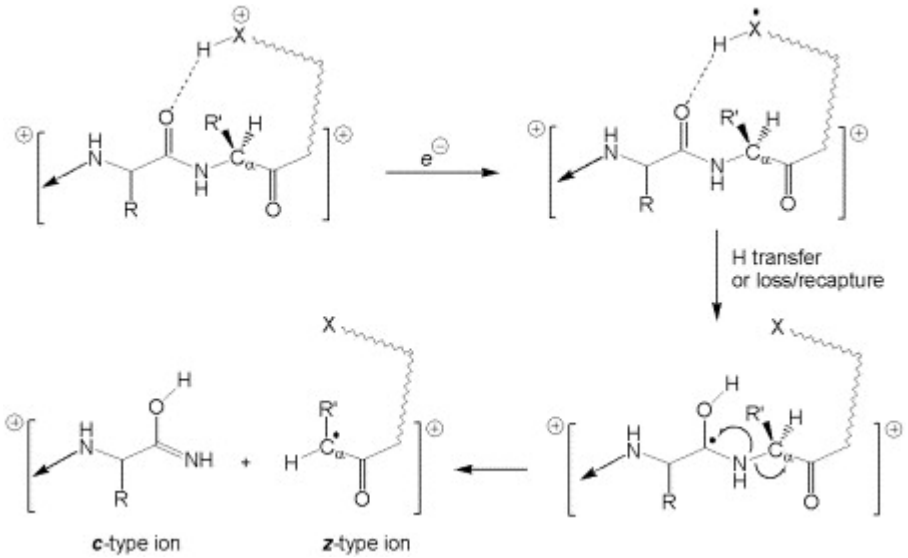
Authors: Syka, JEP; Coon, JJ; Schroeder, MJ; Shabanowitz, J; and Hunt, DF

$$[M + 3H]^{3+} + A^{-} \rightarrow [M + 3H]^{2+\bullet} + A$$

$$[M + 3H]^{2+\bullet} \rightarrow \underbrace{[C+2H]^{1+}}_{C} + \underbrace{[Z+H]^{1+\bullet}}_{Z}$$



ThermoFisher SCIENTIFIC



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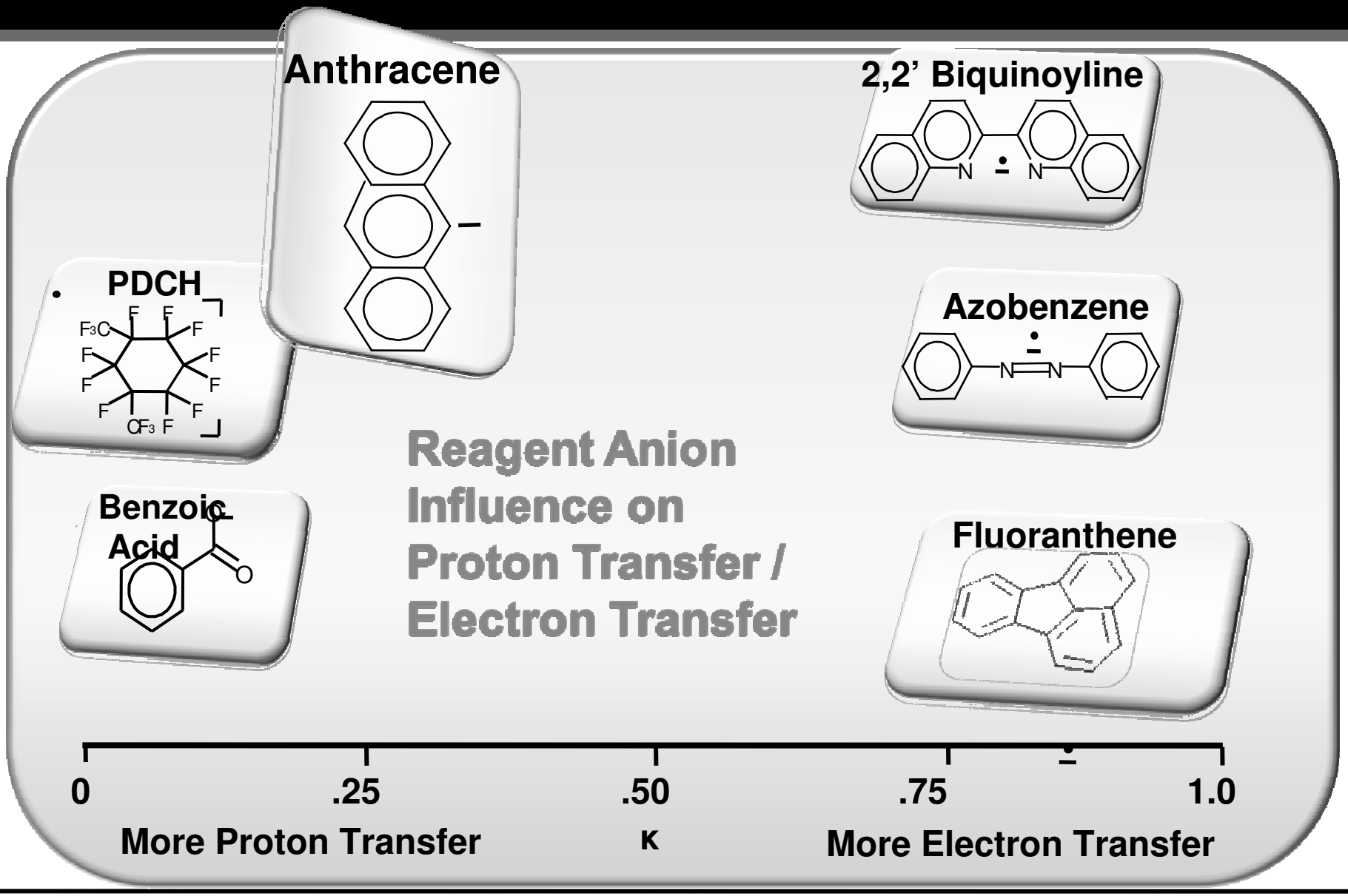
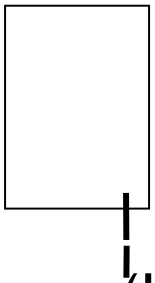
Reagent Anion Influence on Proton Transfer / Electron Transfer



ETD – chemical ionization
ECD

No need of high
vacuum/magnetic field



Available on (Low Cost) Ion Trap Instrument !!!

- Specific information about nature and site of *post-translational modifications* (phosphorylation, glycosylation, oxidation, etc)

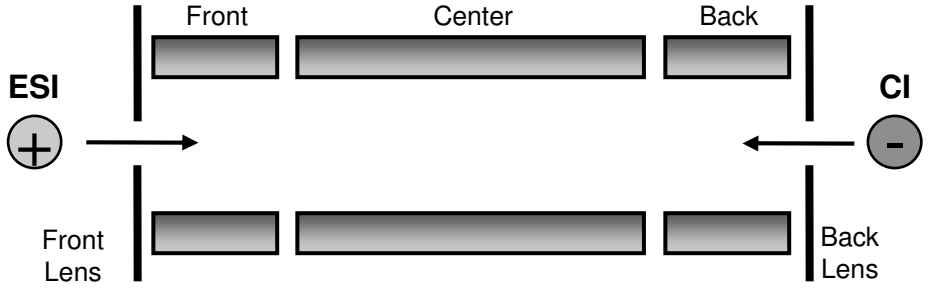
- New capabilities to identify & characterize *very large peptides and intact proteins*

- Analyze *new peptide classes*: non-tryptic highly basic peptides, MHC antigenic peptides, histones

- Dramatically increased *sequence coverage* for proteins

CID		ETD	
Thr, Ser	Loss of water -18 amu, intense for terminal Thr	Thr, Ser	No water losses
Met /MetSO	Loss of CH_3SH (-48 AMU)/ CH_3SOH (-64 AMU)	Met /MetSO	No loss of CH_3SH / CH_3SOH
Ser(PO_4),Thr(PO_4)	Loss of H_3PO_4 (-98 AMU)		No loss of H_3PO_4
Pro	Cleaves easily at on its N-terminal side, resulting in dominant y-type ions & suppressed b-type ions	Pro	No fragmentation on its N-terminal side
O-linked glycosylation	Loss of glycosylation	*O-linked glycosylation*	*No loss or partial loss of glycosylation, peptide backbone fragmentation*
N-linked glycosylation	loss of N-glycosylation	*N-linked glycosylation*	*No loss or partial loss of glycosylation, peptide backbone fragmentation*

Optimally implemented on the high capacity, segmented, linear ion-trap



- High-capacity linear ion trap → Increased sensitivity
- Segmented design w/AGC → Precise control of the ion/ion reaction
- Quadrupole mass filter → Purity of the ETD reagent
- Fast duty cycle → ~ 3 ETD/sec

Routine, fast, sensitive alternating CID & ETD during LC/MS^n

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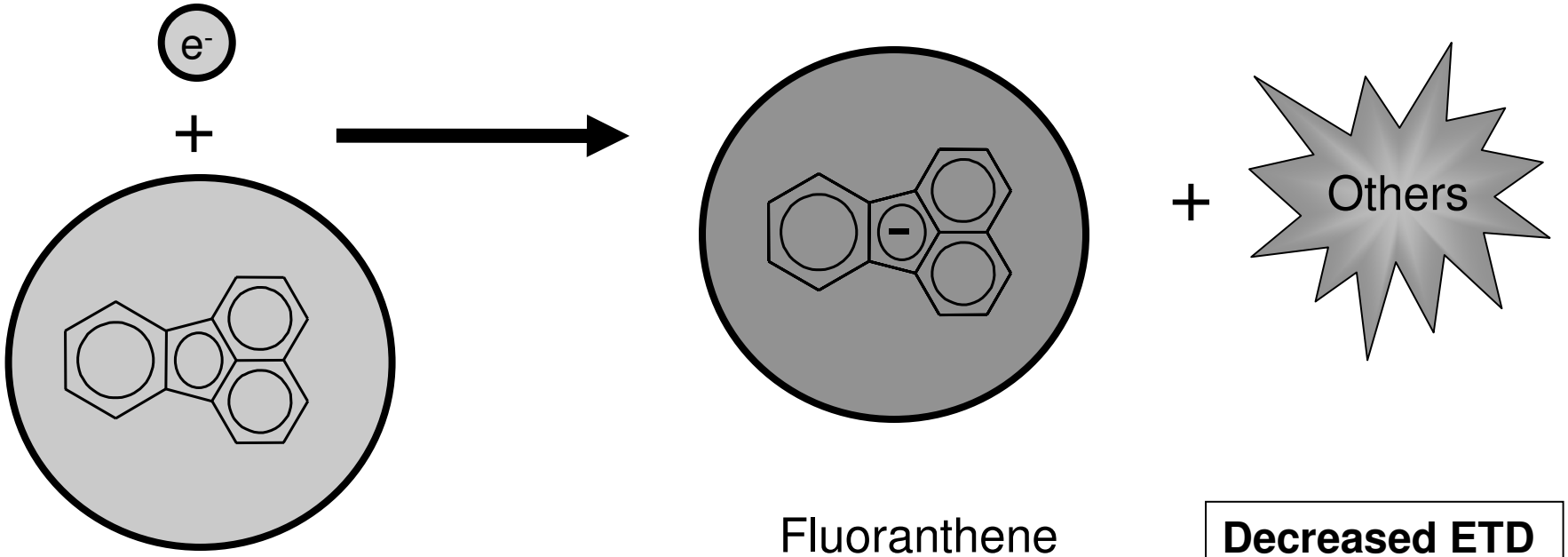
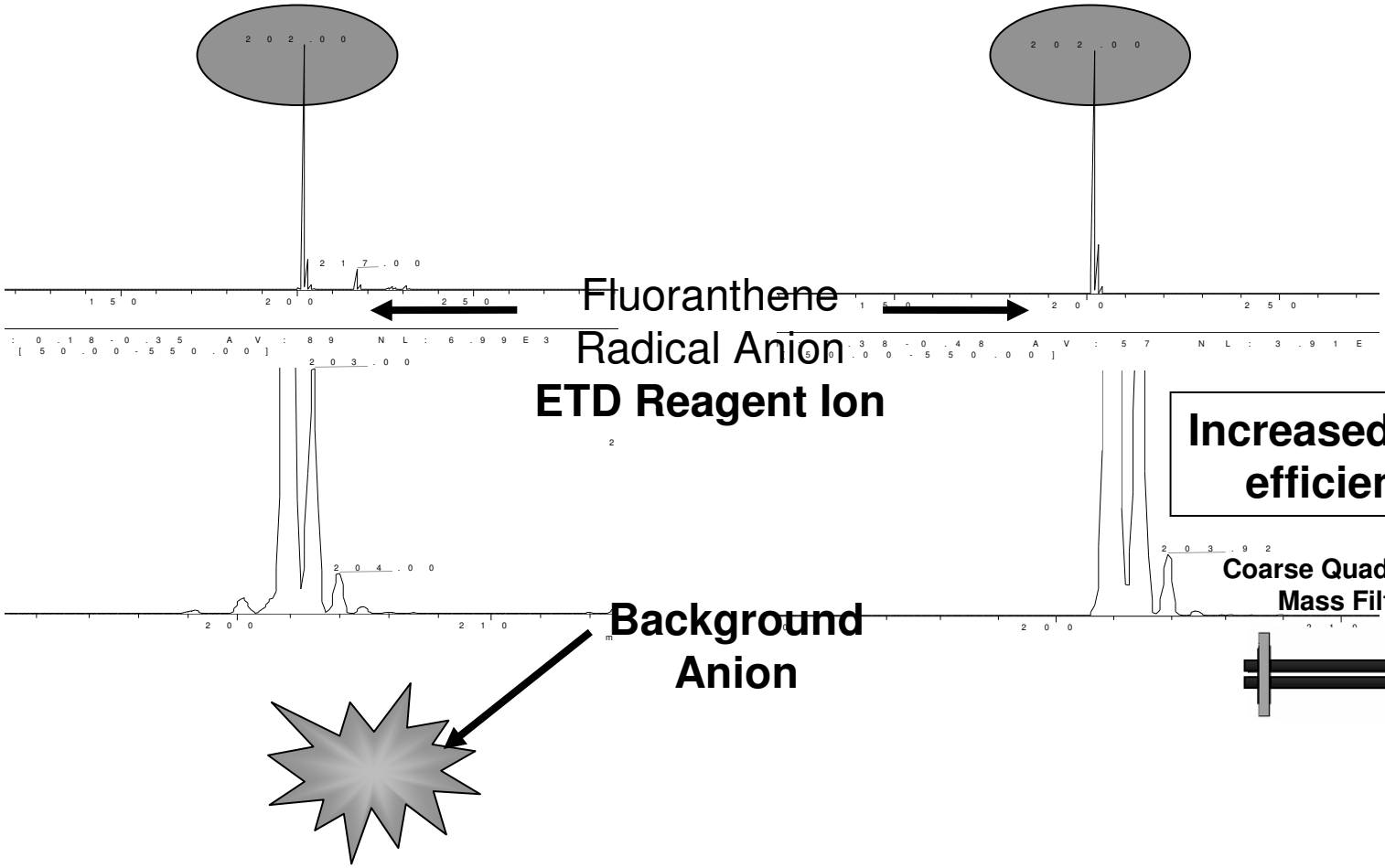
Thermal electrons react with fluoranthene creating the 'ETD reagent'





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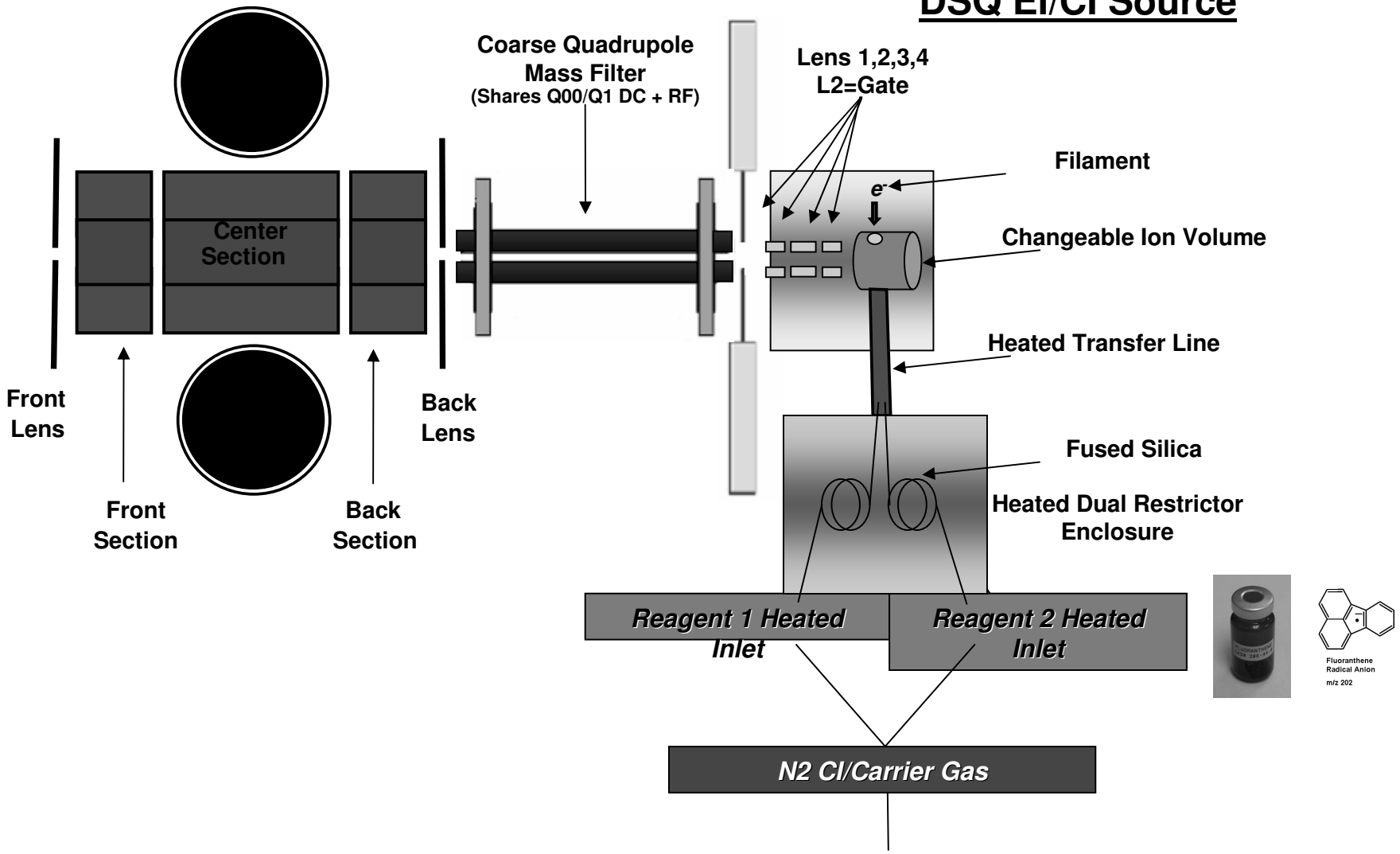
DSQ EI/CI Source



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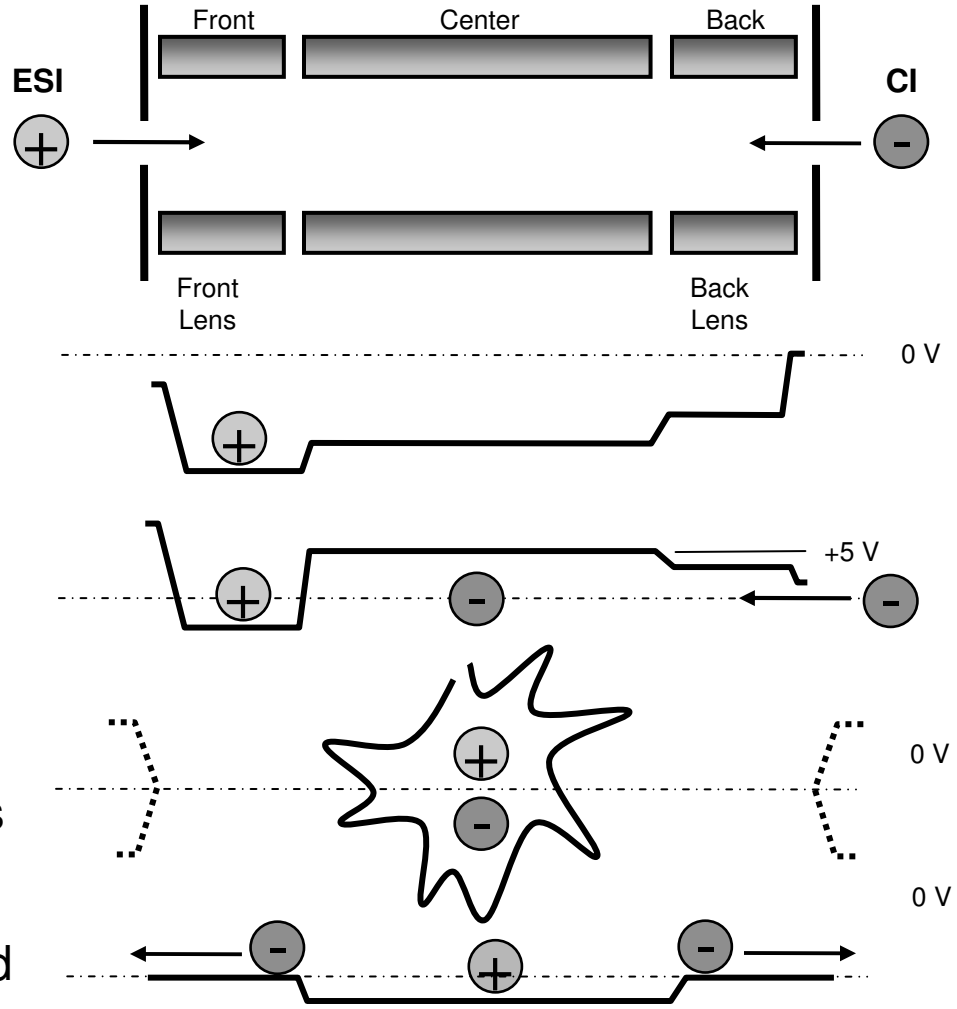
Step 1: Trap and isolate Cations in center, then move them to front section

Step 2: Anion Injection

Step 3: Ion/Ion Reaction – new charge-reduced species created

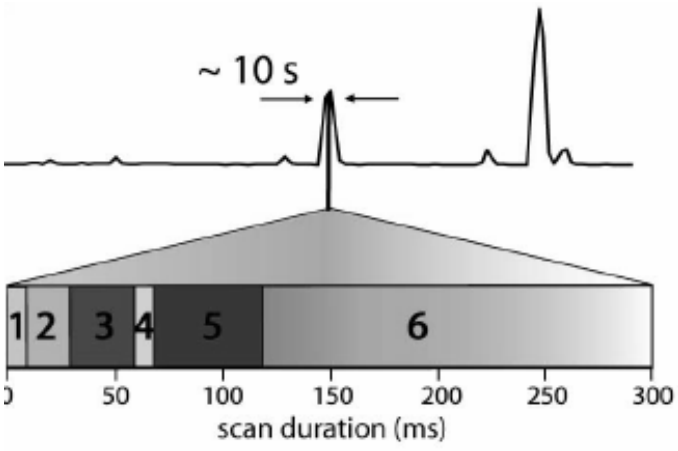
Step 4: Remove any un-reacted anions; scan



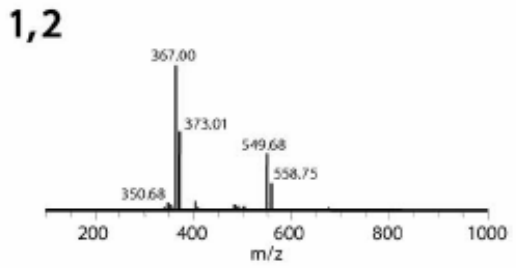
Rapid alternating between CID & ETD during LC/MS^n

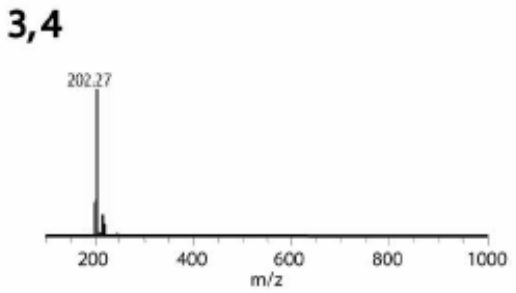
ThermoFisher SCIENTIFIC

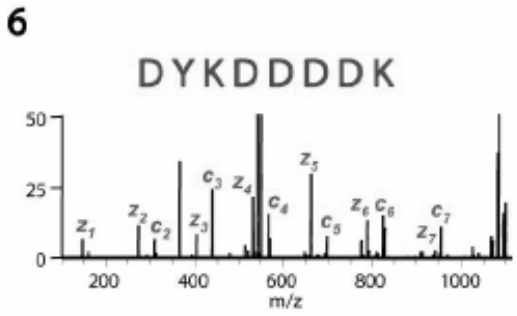
- Precise control of reaction parameters due to Ion Segregation and AGC
- Readily Handles Multiple Reagents and Reaction Types
- High Ion/Charge Capacity is required for sensitive ETD
- Rapidly alternating, sensitive CID and ETD during LC/MS for complementary sequence information



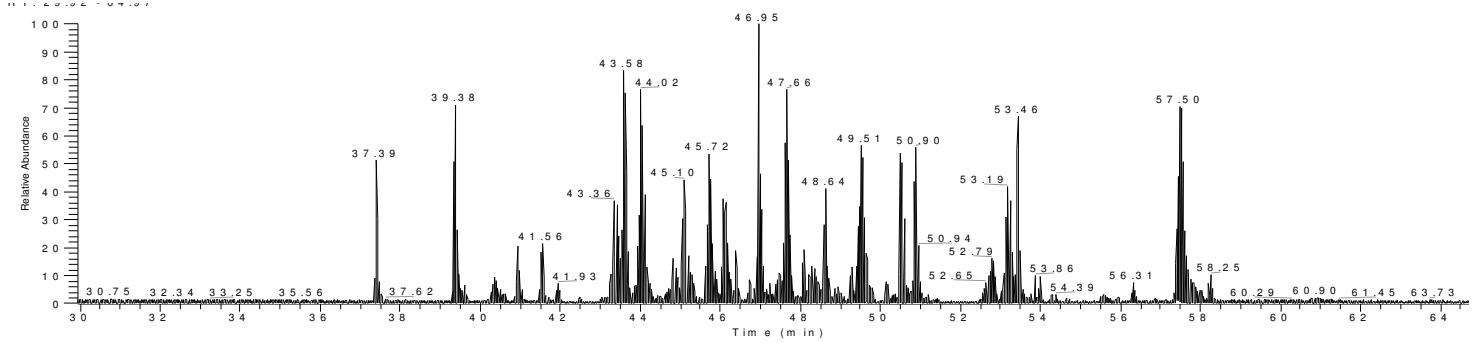
		time (ms)
1	Cation injection	1 - 10
2	Precursor isolation/storage	20
3	Anion injection	1 - 5
4	Anion isolation	10
5	Ion/ion reaction	10 - 50
6	Scan products	200





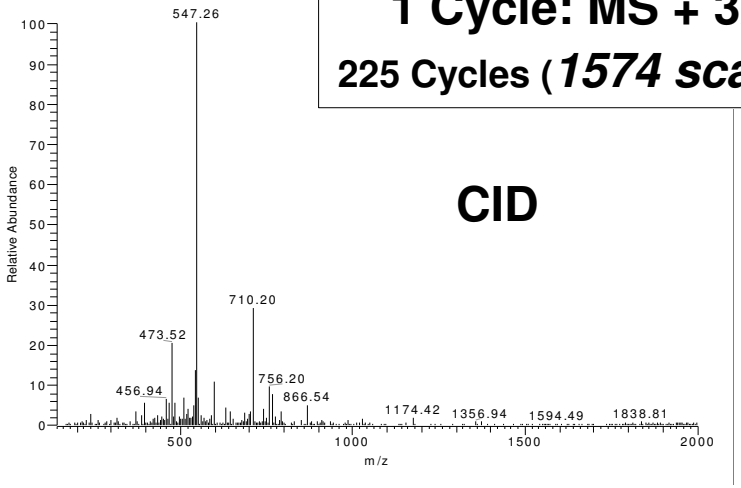


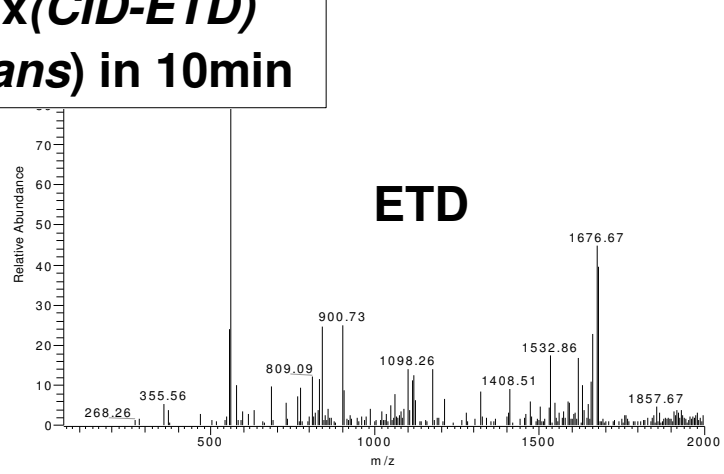
ETD is fast, < 350 ms, so CID and ETD scans in the same method



1 Cycle: MS + 3x*(CID-ETD)*

225 Cycles (*1574 scans*) in 10min





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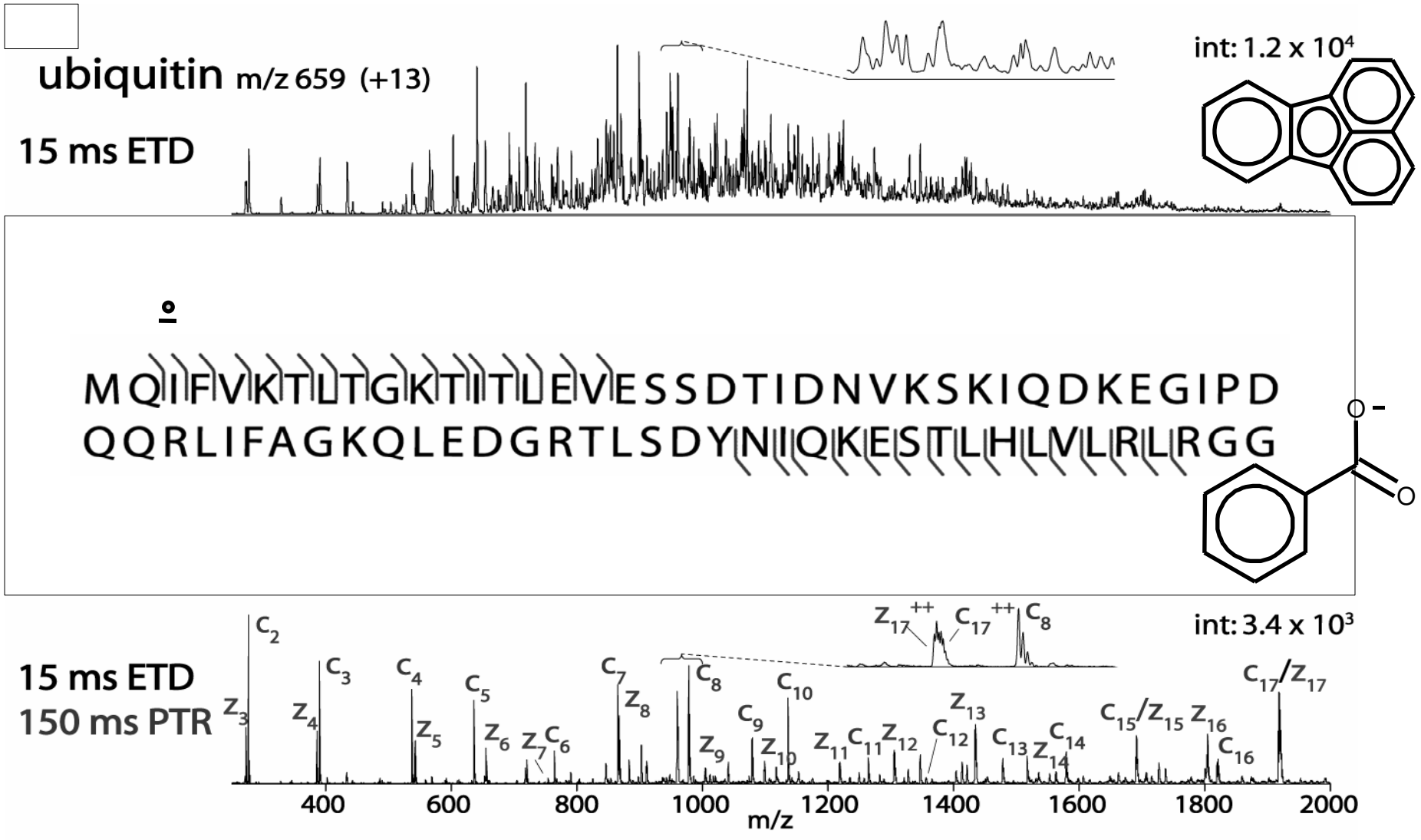
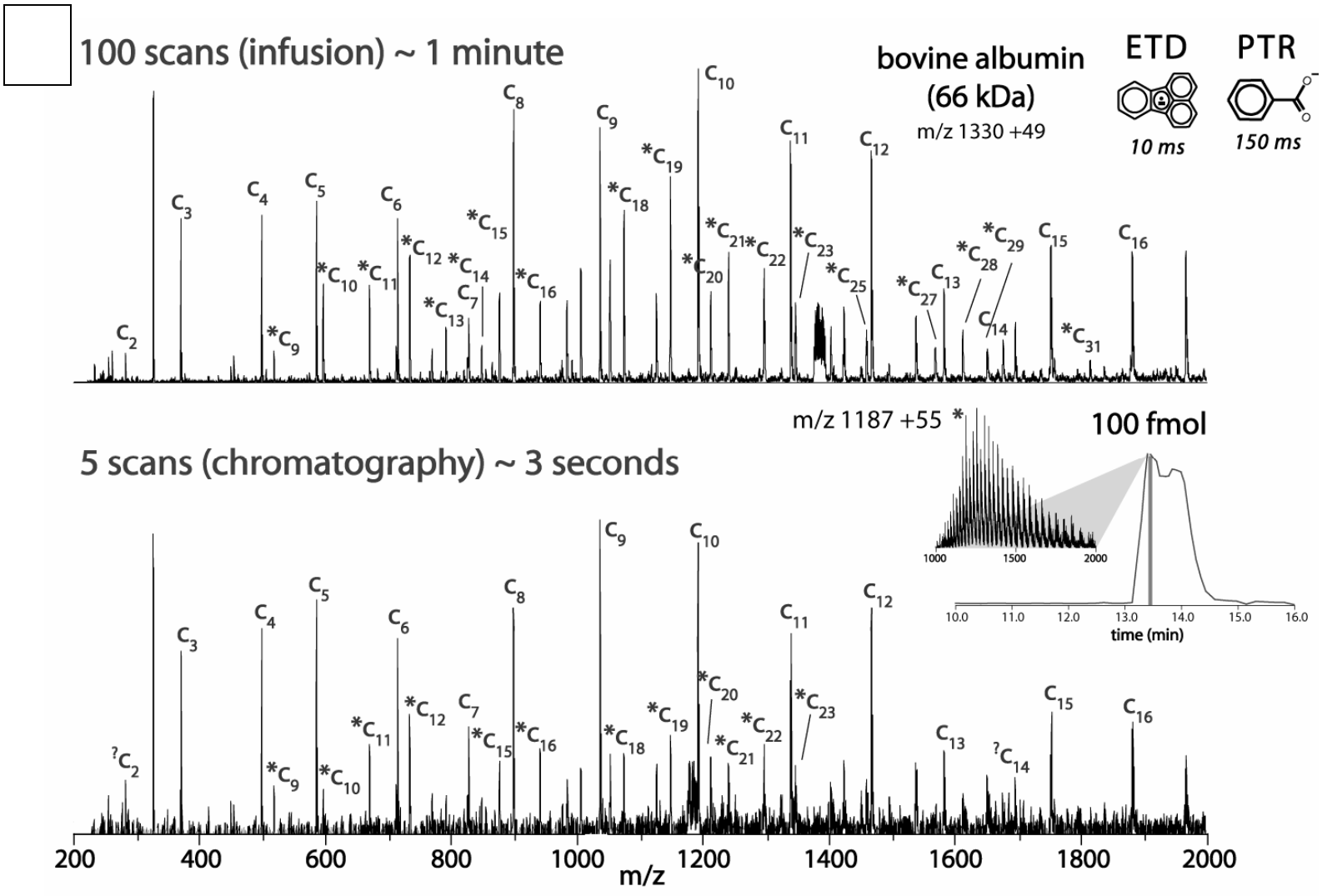


Data courtesy of Coon Group, University of Wisconsin-Madison

ThermoFisher SCIENTIFIC



Data courtesy of Hunt Group, University of Virginia

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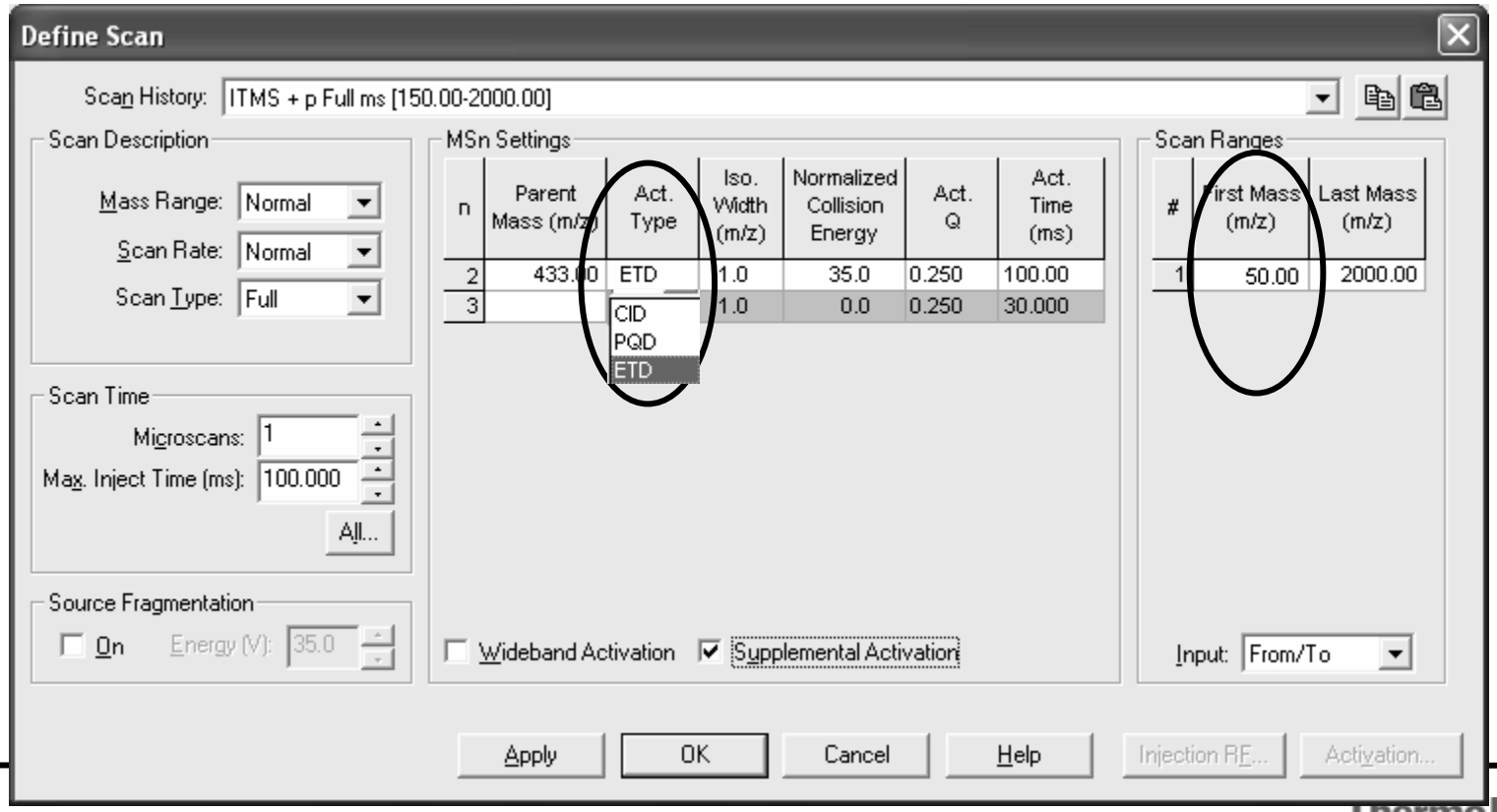
- As Simple to Use as CID and PQD
  - Just Another Dissociation Technique
  - Fully Automatic
  - Well controlled and optimized parameters



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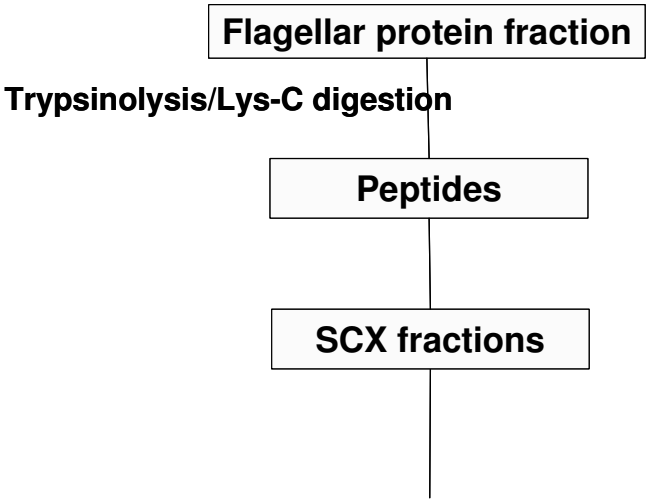
ETD Observations

Effect of charge state

ETD fragmentation rules

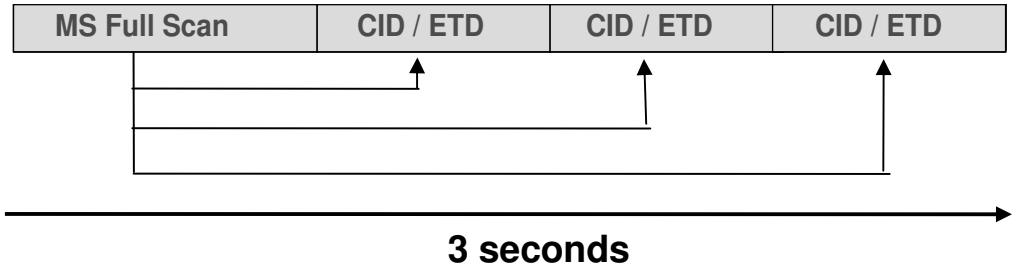
ETD x ECD

Supplemental activation for 2+ ions

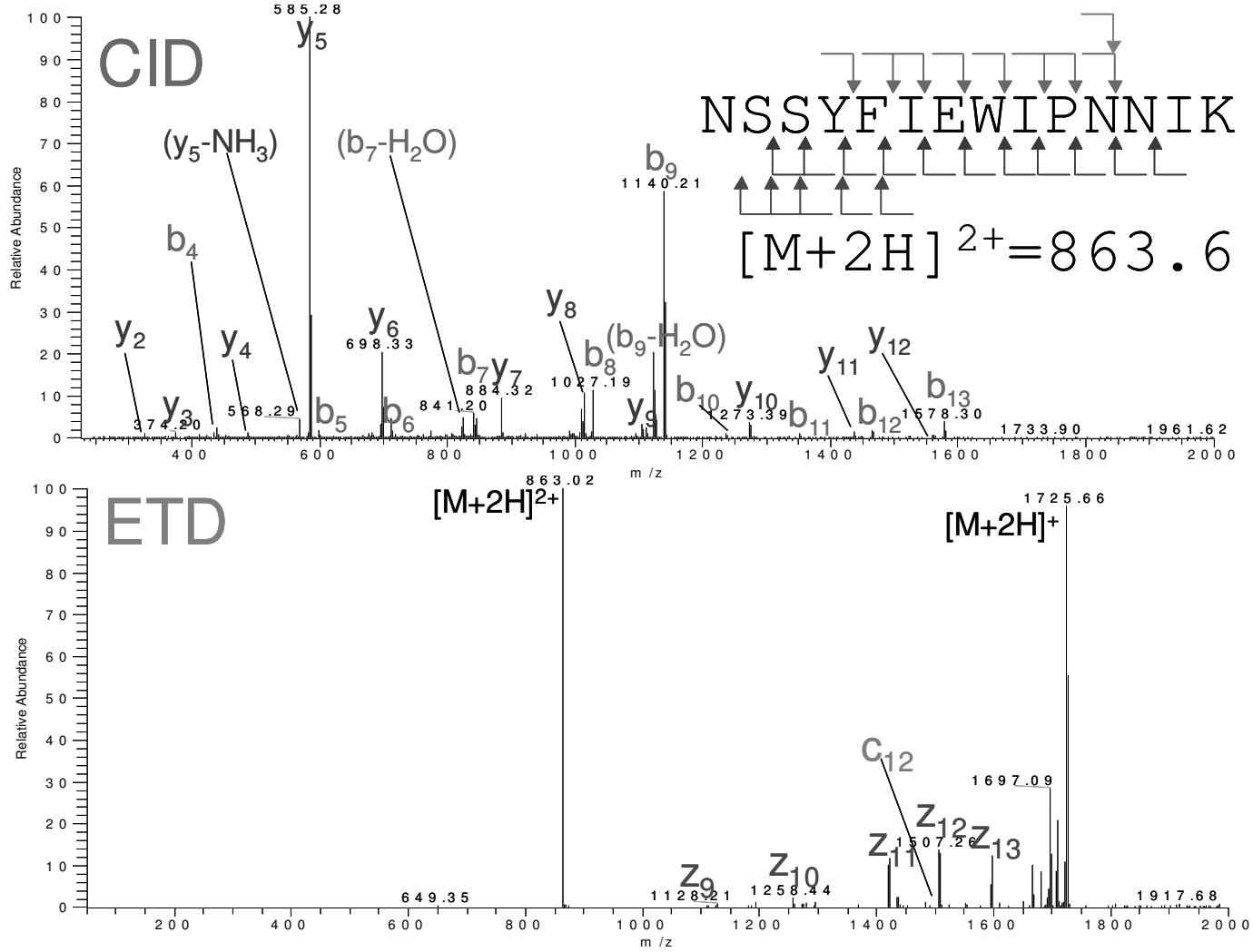


ETD Double Play Method

Alternating CID and ETD on top 3 precursors

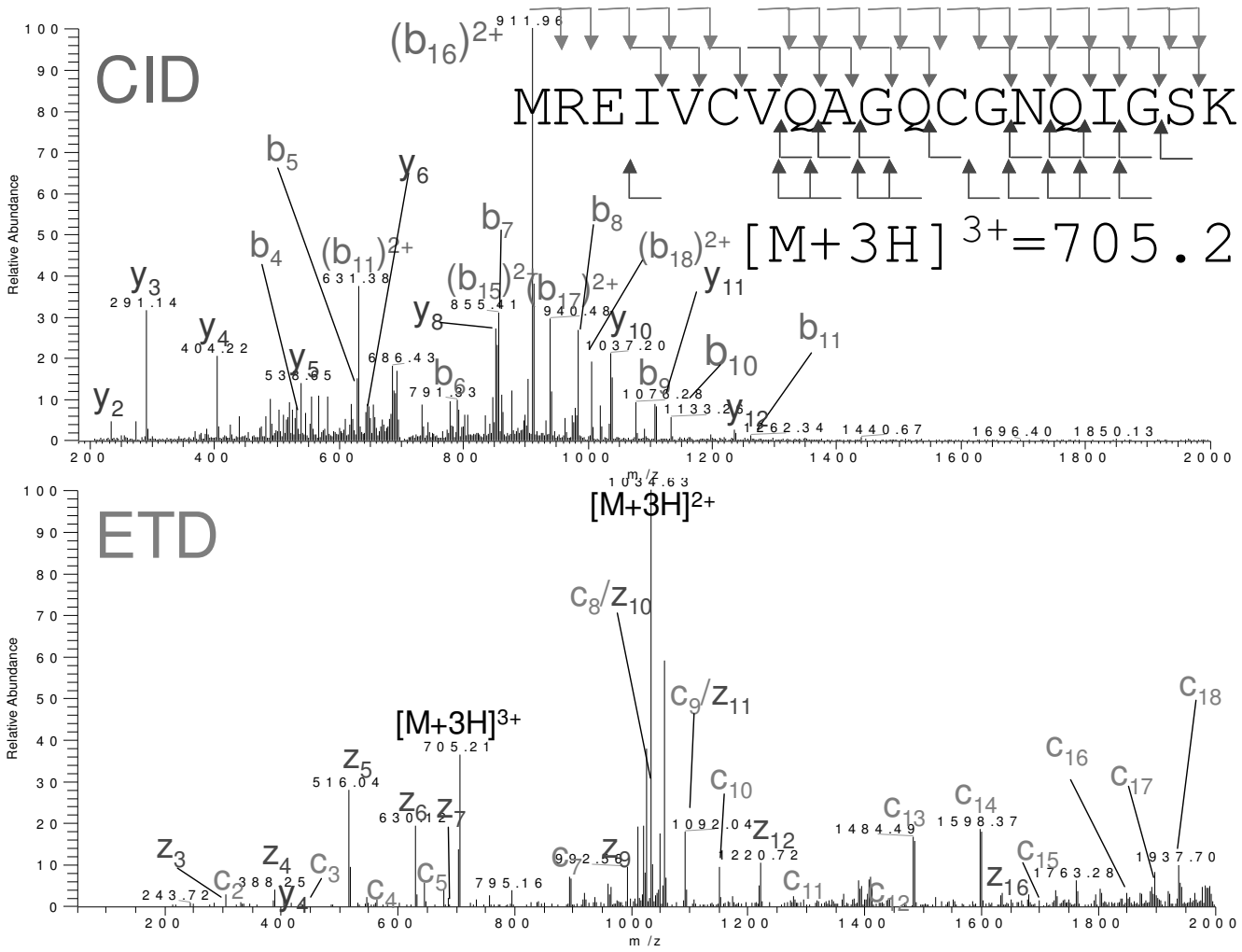


Data courtesy of Sarah Hart, University of Manchester



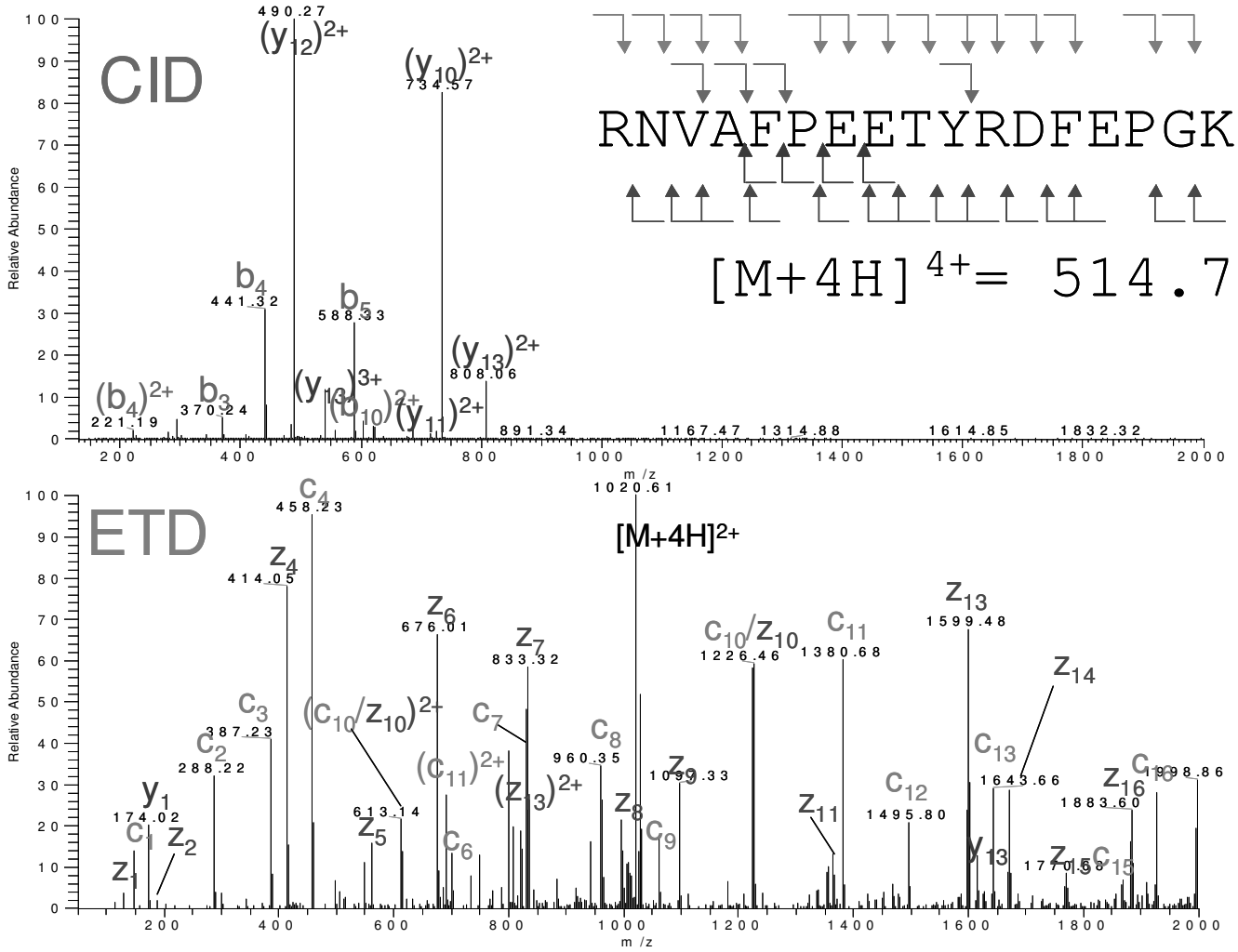
Data courtesy of Sarah Hart, University of Manchester

ThermoFisher SCIENTIFIC



Data courtesy of Sarah Hart, University of Manchester

ThermoFisher SCIENTIFIC



Data courtesy of Sarah Hart, University of Manchester

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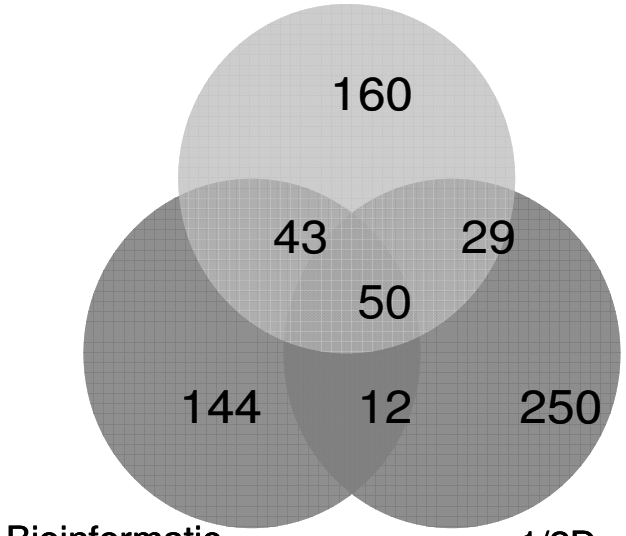
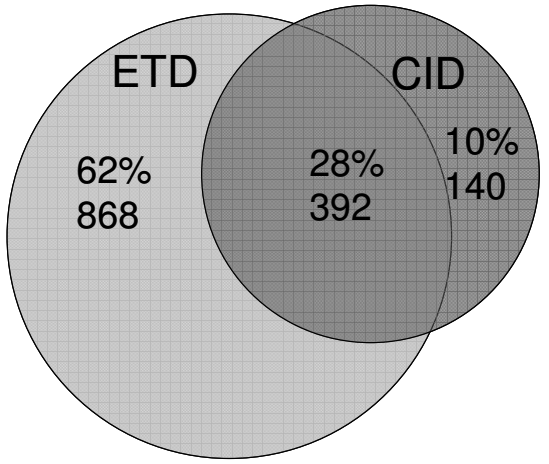
Previous Studies: 688 proteins



This Study: 1400 proteins



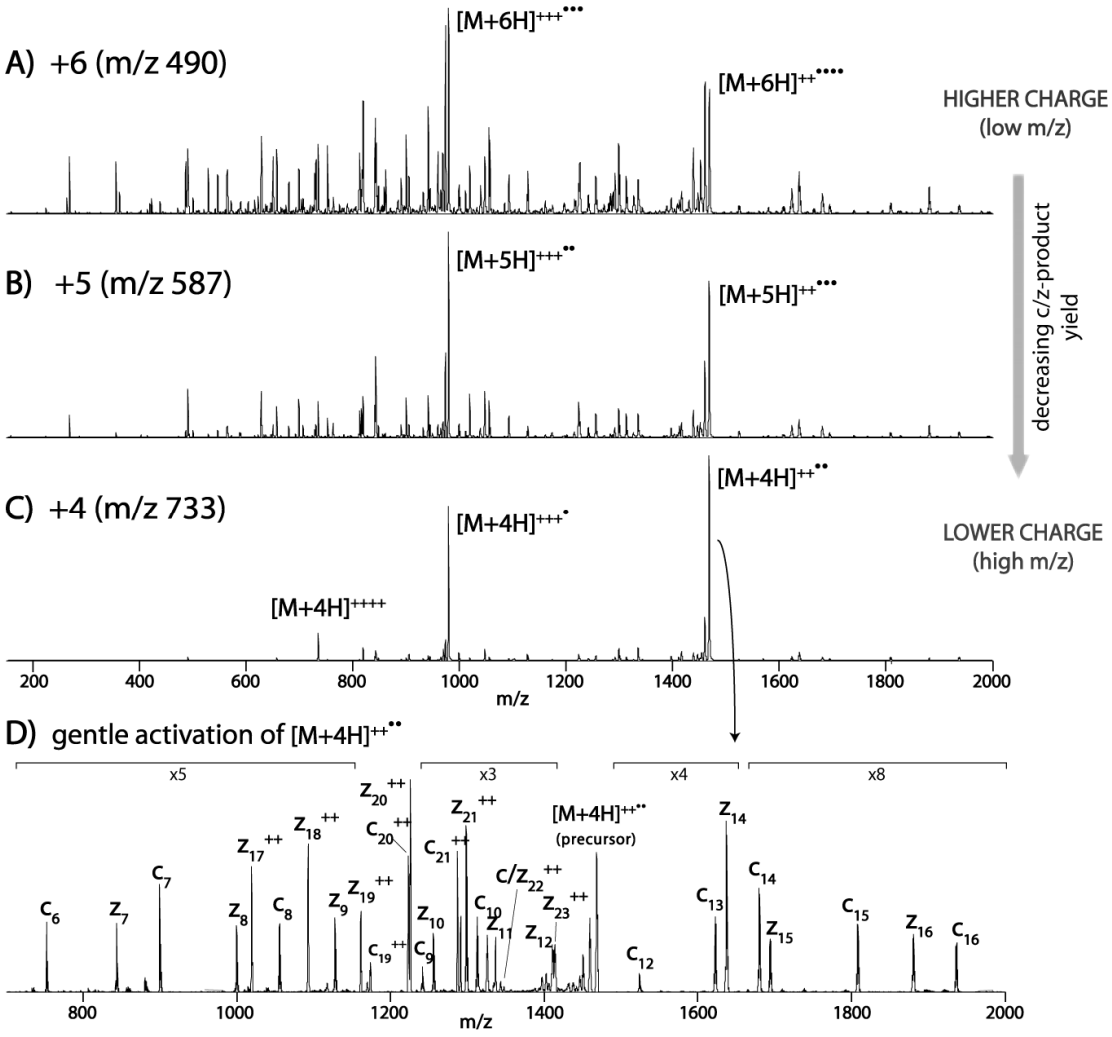
Trypsin
1400 proteins,
20018 peptides

≥2 distinct peptides
Filtered for peptide Charge State vs. XCorr

Increased protein coverage with ETD and CID combined

Data courtesy of Sarah Hart, University of Manchester

ThermoFisher SCIENTIFIC



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- Pairs of related scan from CID and ETD experiment provide direct and unambiguous confirmation of results.
  - CID confirms ETD or ETD confirms CID results
- ETD scores, but CID spectrum does not
  - CID spectrum can simply not interpreted
- CID scores, but ETD spectrum does not
- Improved fragment ion sequence coverage for each peptide
- Increased confidence in protein identification

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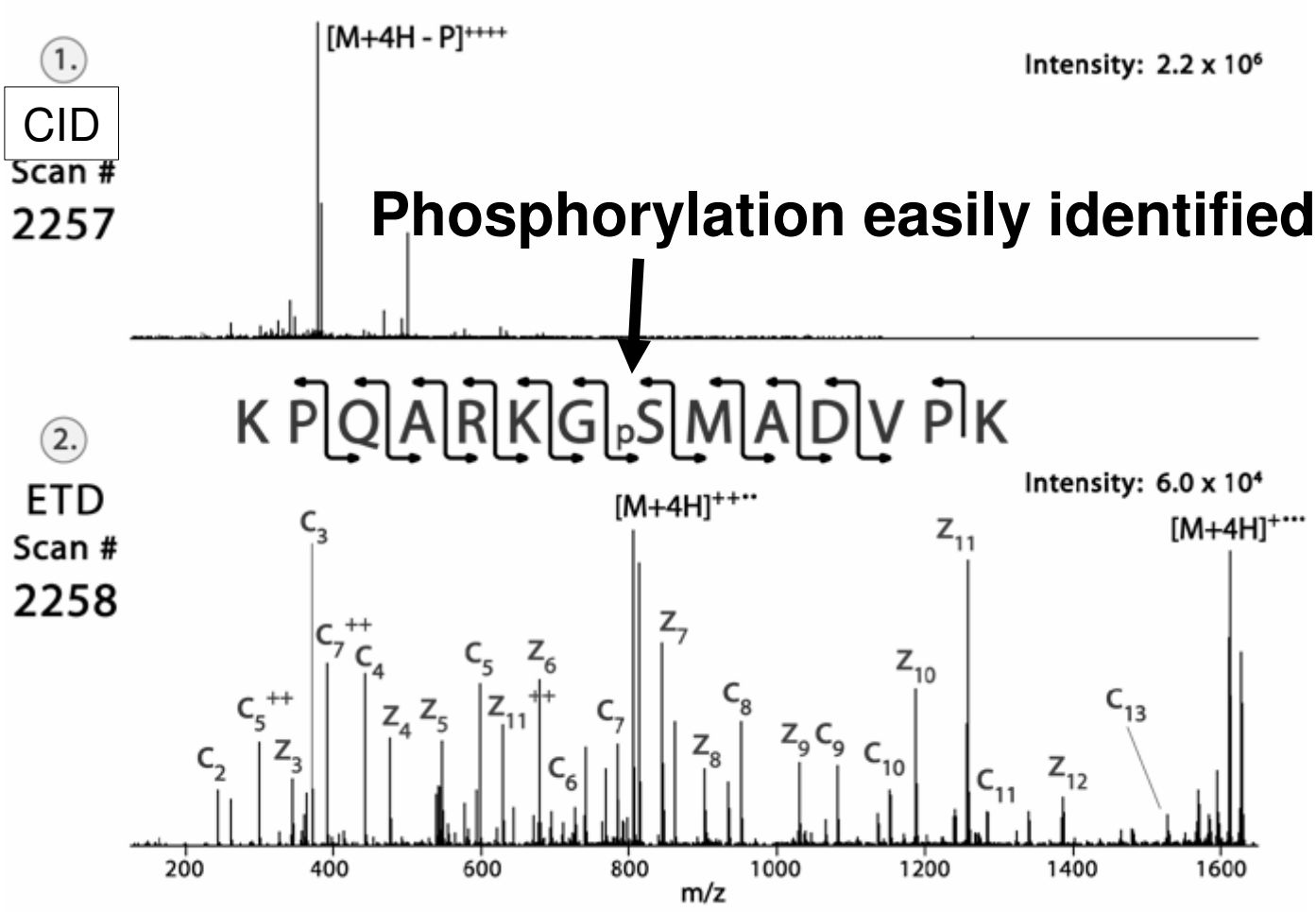
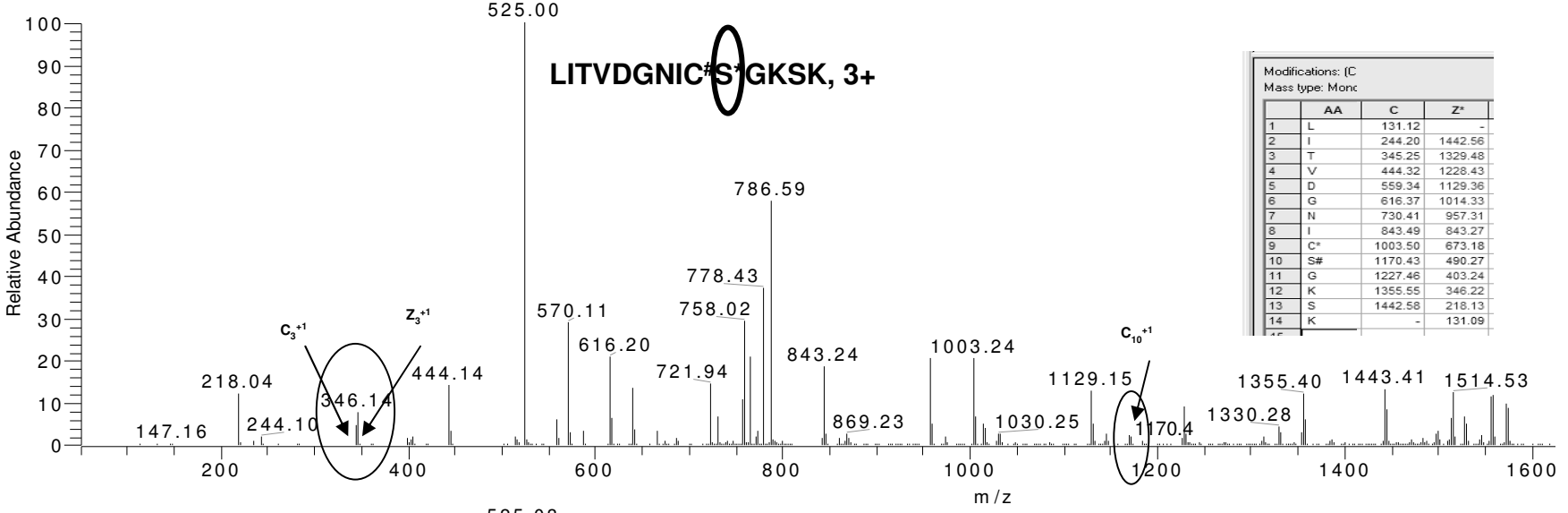
Practical Applications of Electron Transfer Dissociation

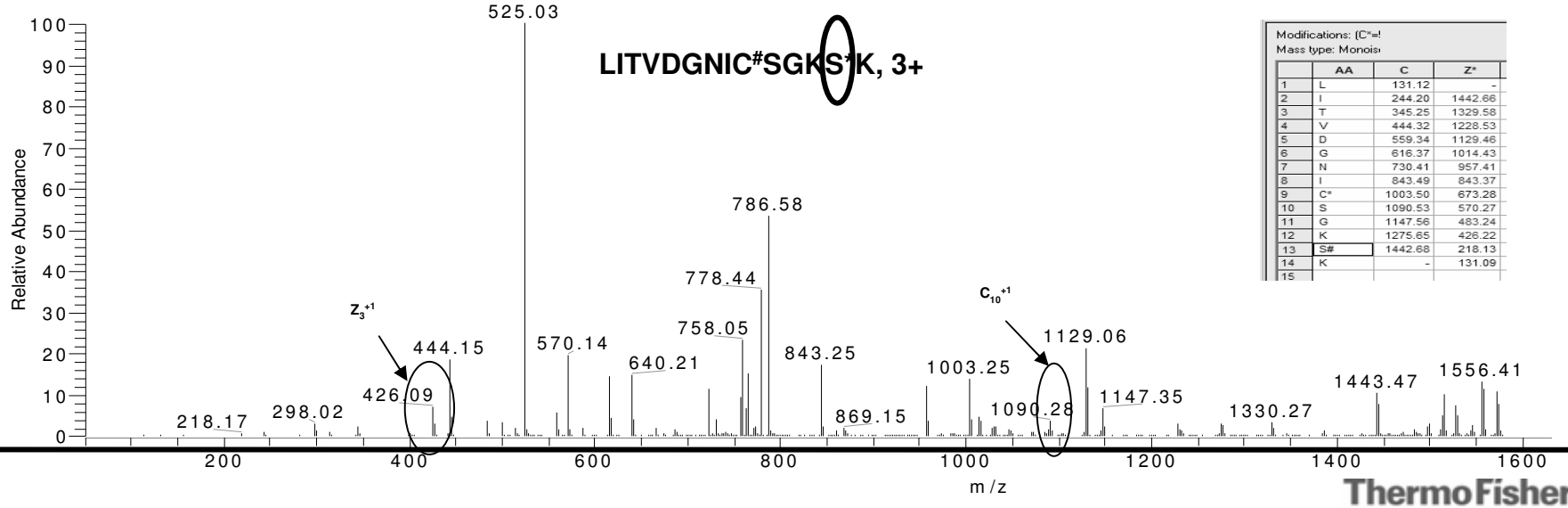
Preservation of labile PTMs

CID/ETD of yeast phosphopeptides



Two phosphopeptides – same sequence, different PTM site



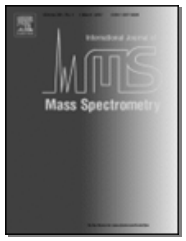


ThermoFisher SCIENTIFIC

The Utility of ETD Mass Spectrometry in Proteomic Analysis

Biochim. Biophys. Acta, 1764, 1811-1822, ***2006***

Authors: Mikesh, LM; Ueberheide, B; Chi, A; Coon, JJ; Syka, JEP; Shabanowitz, J; and Hunt, DF



Analysis of Intact Proteins on a Chromatographic Time Scale by ETD Tandem MS

International Journal of Mass Spectrometry 259, 197-203, ***2007***

Authors: Chi, A; Bai, DL; Geer, LY; Shabanowitz, J; and Hunt, DF

analytical chemistry

Supplemental Activation Method for High-Efficiency ETD of Doubly Protonated Peptide Precursors

Analytical Chemistry, 79, 477-485, ***2007***

Authors: Swaney, DL; McAlister, GC; Wirtala, M; Schwartz, JC; Syka, JEP; and Coon, JJ

PNAS

Analysis of Phosphorylation Sites on Proteins from Saccharomyces Cerevisiae by ETD MS/MS

Proc. Natl. Acad. Sci. USA, 104, 2193-2198, ***2007***

Authors: Chi, A; Huttenhower, C; Geer, LY; Coon, JJ; Syka, JEP; Bai, DL; Shabanowitz, J; Burke, DJ; Troyanskaya, OG; and Hunt, DF

ThermoFisher SCIENTIFIC