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Intact Protein Analysis Using Electron Transfer Dissociation Mass Spectrometry

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San Jose, CA

High Resolution, Accurate Mass, ETD

SCIENTIFIC

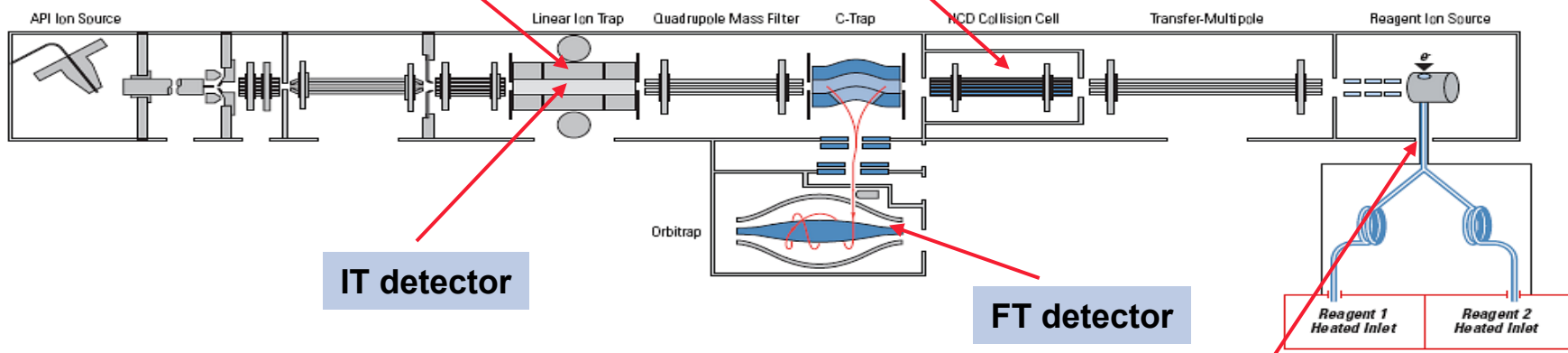
MINIREVIEW

Top-down MS, a powerful complement to the high capabilities of MS/MS analysis proteomic

CID & ETD

HCD

Fred W. McLafferty¹, Kathrin Breuker², Mi Jin¹, Xuemei Han¹, Giuseppe Infusini¹, Honghai Jiang¹



Electron-transfer dissociation [30], works well with less sensitive MS instruments, and can be applied to peptides and smaller proteins [31] with versatile ion-ion collisions [32]. Of special promise for routine top-down applications is the recently developed Orbitrap mass spectrometer, which has resolution and mass accuracy capabilities approaching those of FT MS, with very promising cost advantages [33]. ECD and electron-transfer dissociation are less sensitive than CAD or IPD, in part because they produce far more product ions.

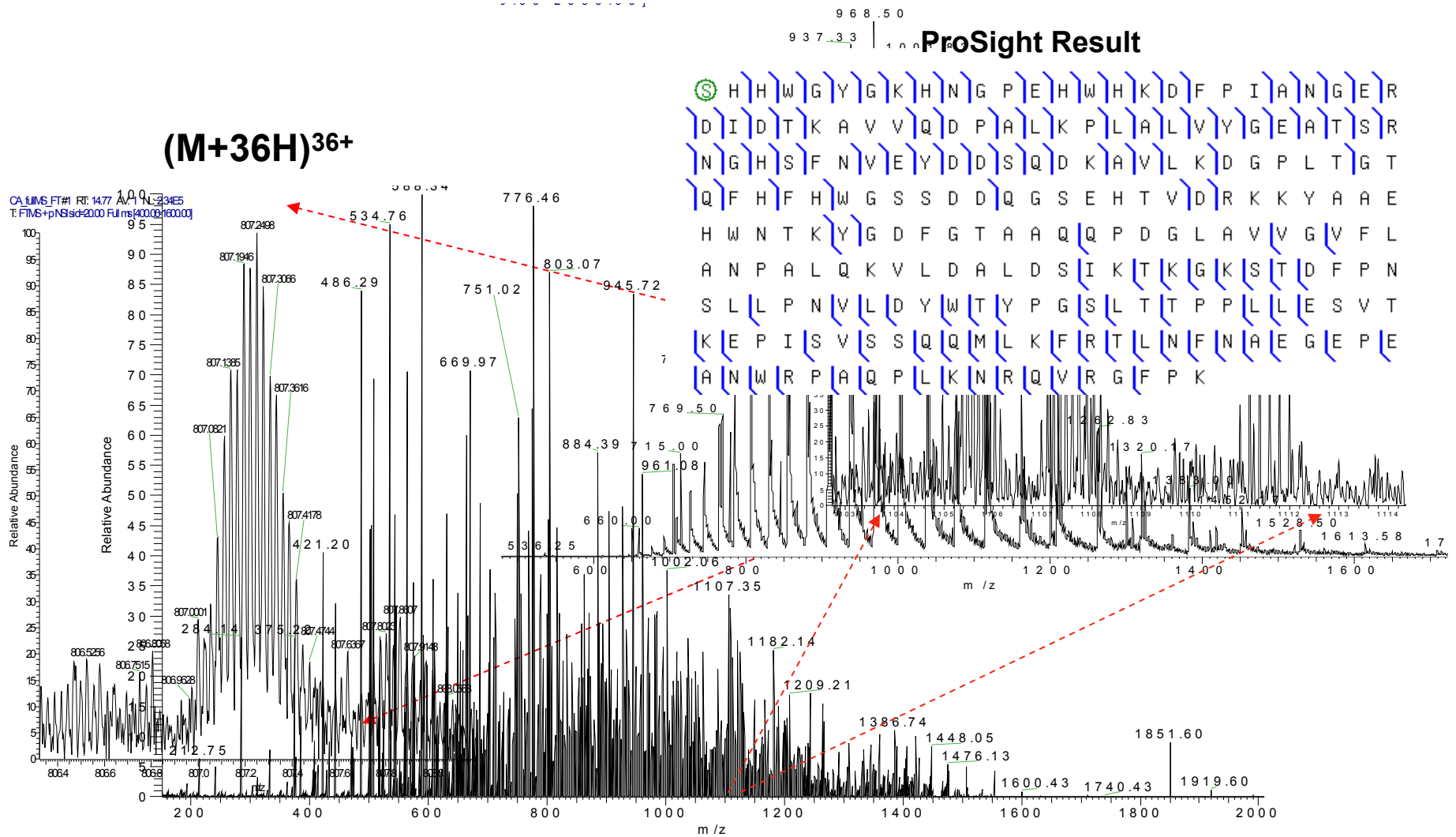


Reagent ion source

Intact Protein Sequencing Using ETD

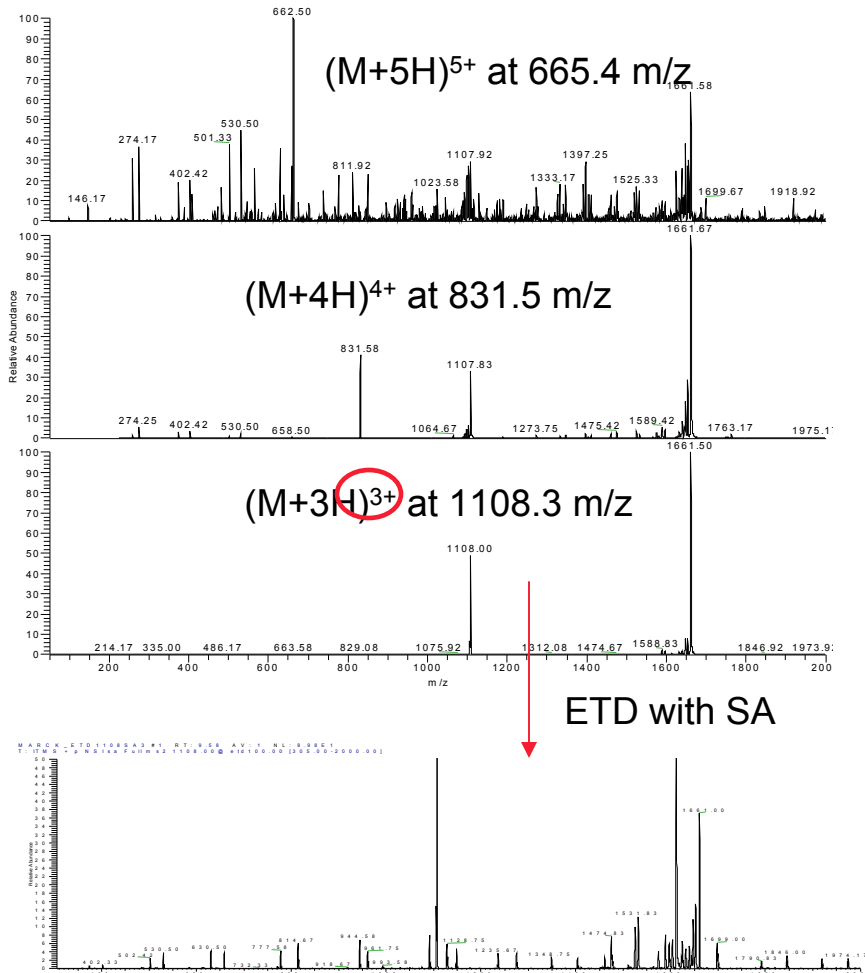
- With Orbitrap detection
- The challenge and issue for data analysis
- Without high resolution (ion trap detection)
- The limitation of unit resolution experiment

ETD of Carbonic Anhydrase (29 KDa) with Orbitrap Detection



Precursor Charge State and ETD Performance

KKKKKRFsFKKsFKLSGFsFKKNNK
3322 Da



Carbonic Anhydrase
29 KDa

(M+36H)³⁶⁺ at 807.7 m/z

S H H W G Y G K H N G P E H W H K D F P I A N G E R Q S P V
 D I D T K A V V Q D P A L K P L A L V Y G E A T S R R M V N
 N G H S F N V E Y D D S Q D K A V L K D G P L T G T Y R L V
 Q F H F H W G S S D D Q G S E H T V D R K K Y A A E L H L V
 H W N T K Y G D F G T A A Q Q P D G L A V V G V F L K V G D
 A N P A L Q K V L D A L D S I K T K G K S T D F P N F D P G
 S L L P N V L D Y W T Y P G S L T T P P L L E S V T W I V L
 K E P I S V S S Q Q M L K F R T L N F N A E G E P E L L M L
 A N W R P A Q P L K N R Q V R G F P K

(M+26H)²⁶⁺ at 1117.7 m/z

S H H W G Y G K H N G P E H W H K D F P I A N G E R Q S P V
 D I D T K A V V Q D P A L K P L A L V Y G E A T S R R M V N
 N G H S F N V E Y D D S Q D K A V L K D G P L T G T Y R L V
 Q F H F H W G S S D D Q G S E H T V D R K K Y A A E L H L V
 H W N T K Y G D F G T A A Q Q P D G L A V V G V F L K V G D
 A N P A L Q K V L D A L D S I K T K G K S T D F P N F D P G
 S L L P N V L D Y W T Y P G S L T T P P L L E S V T W I V L
 K E P I S V S S Q Q M L K F R T L N F N A E G E P E L L M L
 A N W R P A Q P L K N R Q V R G F P K

Effect of ETD Reaction Time on Sequence Coverage

2 ms

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Ⓢ H H W G Y G K H N G P E H W H K D F P I A N G E R Q S P V  
D I D T K A V V Q D P A L K P L A L V Y G E A T S R R M V N  
N G H S F N V E Y D D S Q D K A V L K D G P L T G T Y R L V  
Q F H F H W G S S D D Q G S E H T V D R K K Y A A E L H L V  
H W N T K Y G D F G T A A Q Q P D G L A V V G V F L K V G D  
A N P A L Q K V L D A L D S I K T K G K S T D F P N F D P G  
S L L P N V L D Y W T Y P G S L T T P P L L E S V T W I V L  
K E P I S V S S Q Q M L K F R T L N F N A E G E P E L L M L  
A N W R P A Q P L K N R Q V R G F P K
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4 ms

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Ⓢ H H W G Y G K H N G P E H W H K D F P I A N G E R Q S P V  
D I D T K A V V Q D P A L K P L A L V Y G E A T S R R M V N  
N G H S F N V E Y D D S Q D K A V L K D G P L T G T Y R L V  
Q F H F H W G S S D D Q G S E H T V D R K K Y A A E L H L V  
H W N T K Y G D F G T A A Q Q P D G L A V V G V F L K V G D  
A N P A L Q K V L D A L D S I K T K G K S T D F P N F D P G  
S L L P N V L D Y W T Y P G S L T T P P L L E S V T W I V L  
K E P I S V S S Q Q M L K F R T L N F N A E G E P E L L M L  
A N W R P A Q P L K N R Q V R G F P K
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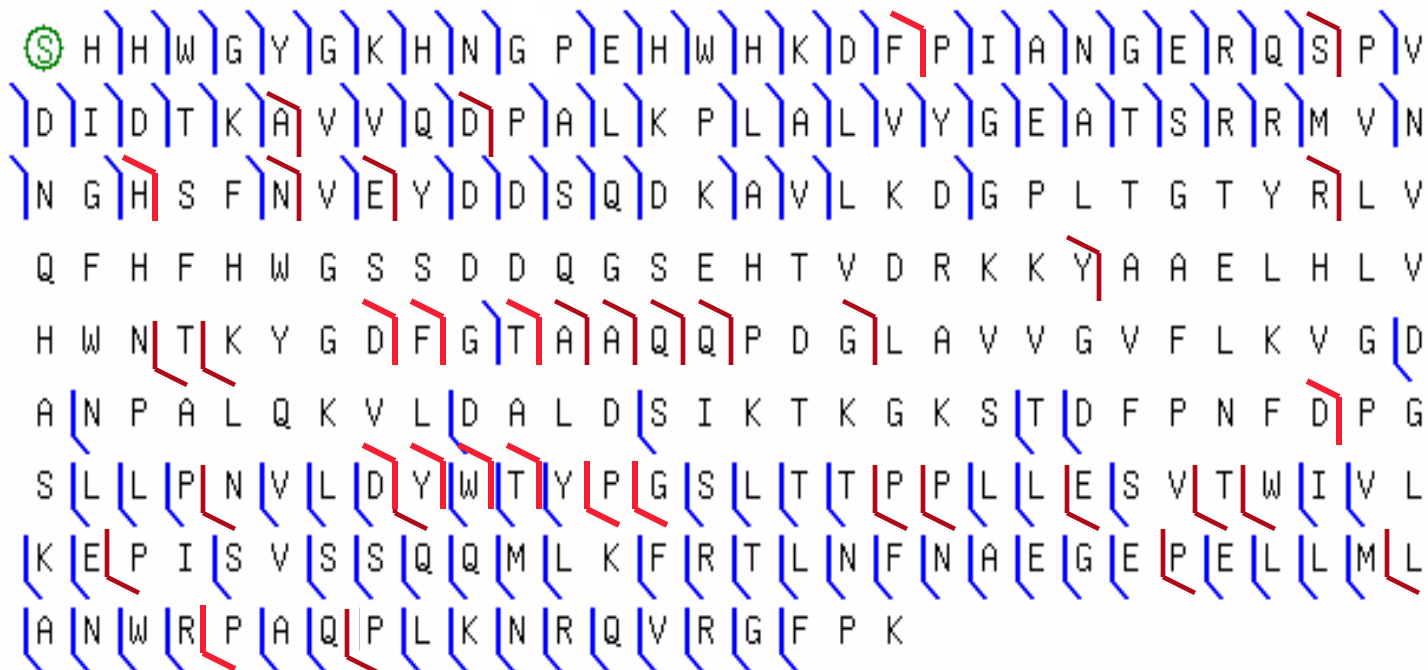
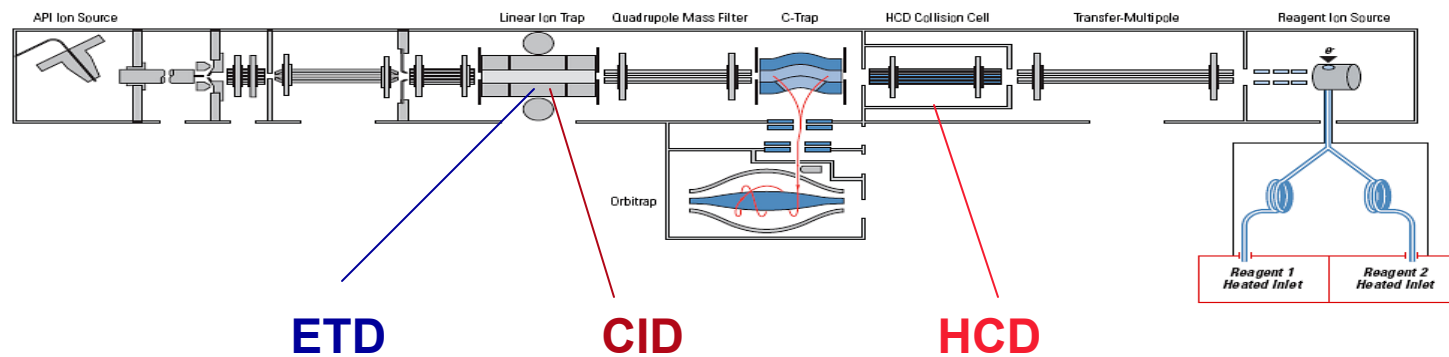
20 ms

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Ⓢ H H W G Y G K H N G P E H W H K D F P I A N G E R Q S P V  
D I D T K A V V Q D P A L K P L A L V Y G E A T S R R M V N  
N G H S F N V E Y D D S Q D K A V L K D G P L T G T Y R L V  
Q F H F H W G S S D D Q G S E H T V D R K K Y A A E L H L V  
H W N T K Y G D F G T A A Q Q P D G L A V V G V F L K V G D  
A N P A L Q K V L D A L D S I K T K G K S T D F P N F D P G  
S L L P N V L D Y W T Y P G S L T T P P L L E S V T W I V L  
K E P I S V S S Q Q M L K F R T L N F N A E G E P E L L M L  
A N W R P A Q P L K N R Q V R G F P K
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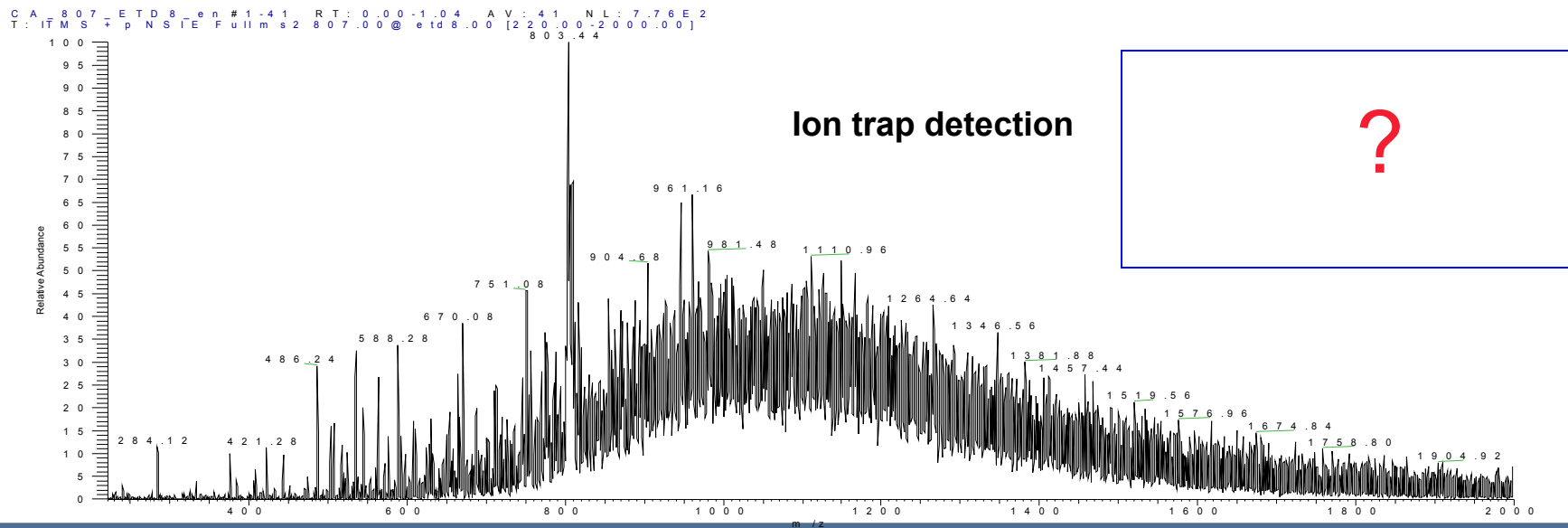
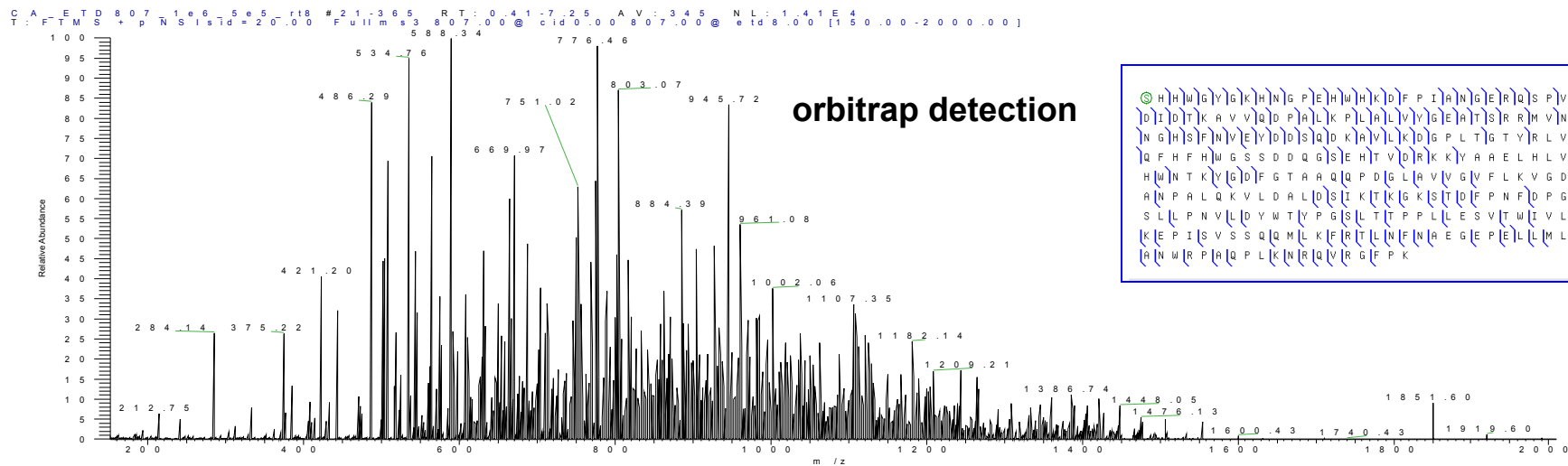
50 ms

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Ⓢ H H W G Y G K H N G P E H W H K D F P I A N G E R Q S P V  
D I D T K A V V Q D P A L K P L A L V Y G E A T S R R M V N  
N G H S F N V E Y D D S Q D K A V L K D G P L T G T Y R L V  
Q F H F H W G S S D D Q G S E H T V D R K K Y A A E L H L V  
H W N T K Y G D F G T A A Q Q P D G L A V V G V F L K V G D  
A N P A L Q K V L D A L D S I K T K G K S T D F P N F D P G  
S L L P N V L D Y W T Y P G S L T T P P L L E S V T W I V L  
K E P I S V S S Q Q M L K F R T L N F N A E G E P E L L M L  
A N W R P A Q P L K N R Q V R G F P K
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Benefiting from Complementary Fragmentation Methods



ETD spectrum on (M+36H)³⁶⁺ of Carbonic Anhydrase



Protein identification using sequential ion/ion reactions and tandem mass spectrometry

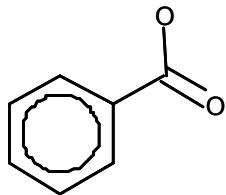
Joshua J. Coon^{*†}, Beatrix Ueberheide^{*}, John E. P. Syka^{*§}, Deanna D. Dryhurst[¶], Juan Ausio[¶], Jeffrey Shabanowitz^{*}, and Donald F. Hunt^{*||**}

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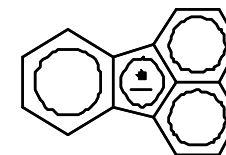
Edited by Fred W. McLafferty, Cornell University, Ithaca, NY, and approved May 24, 2005 (received for review April 19, 2005)

Electron Transfer and Proton Transfer

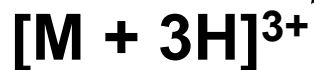
Benzoic Acid



Fluoranthene



Proton Transfer

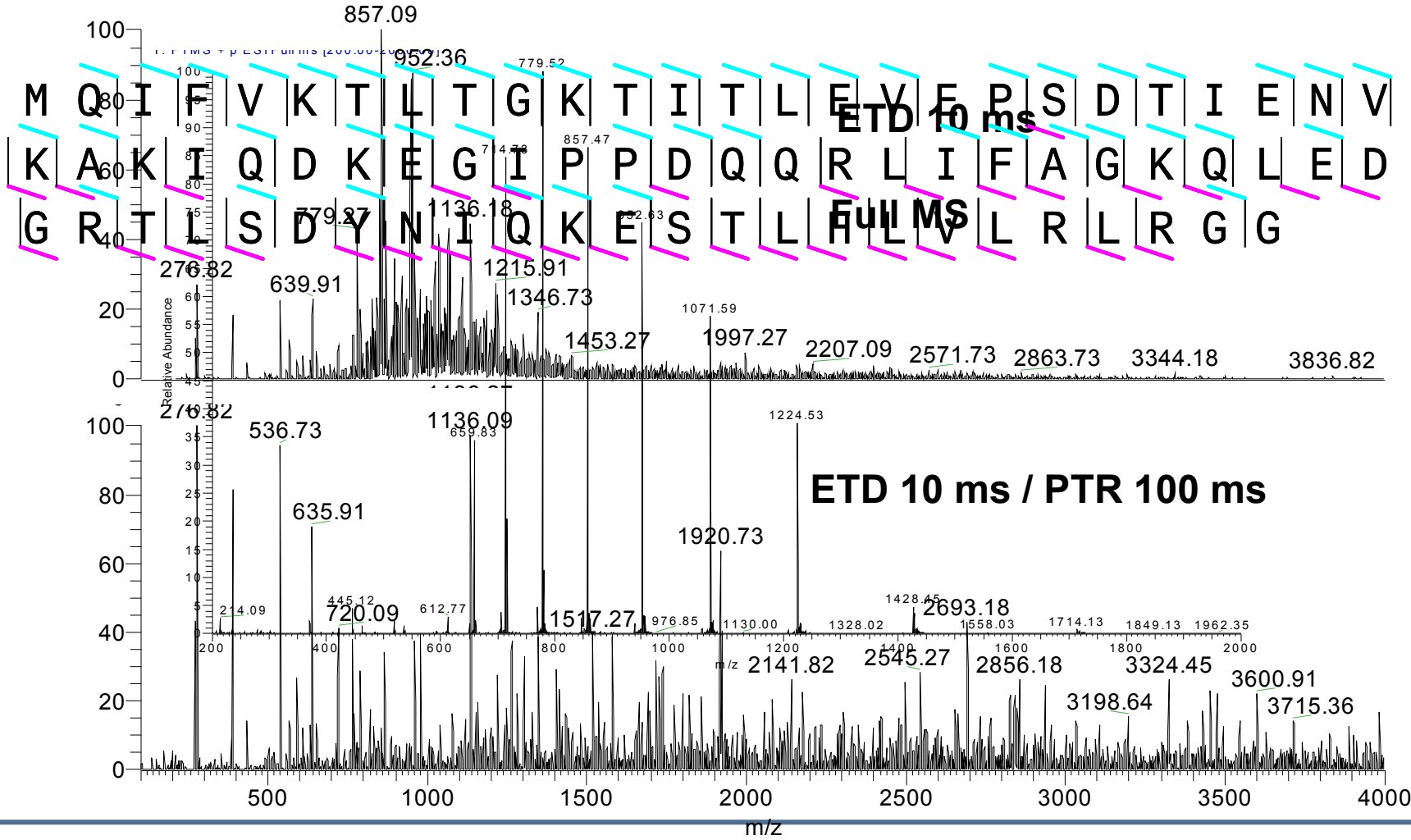


Electron Transfer

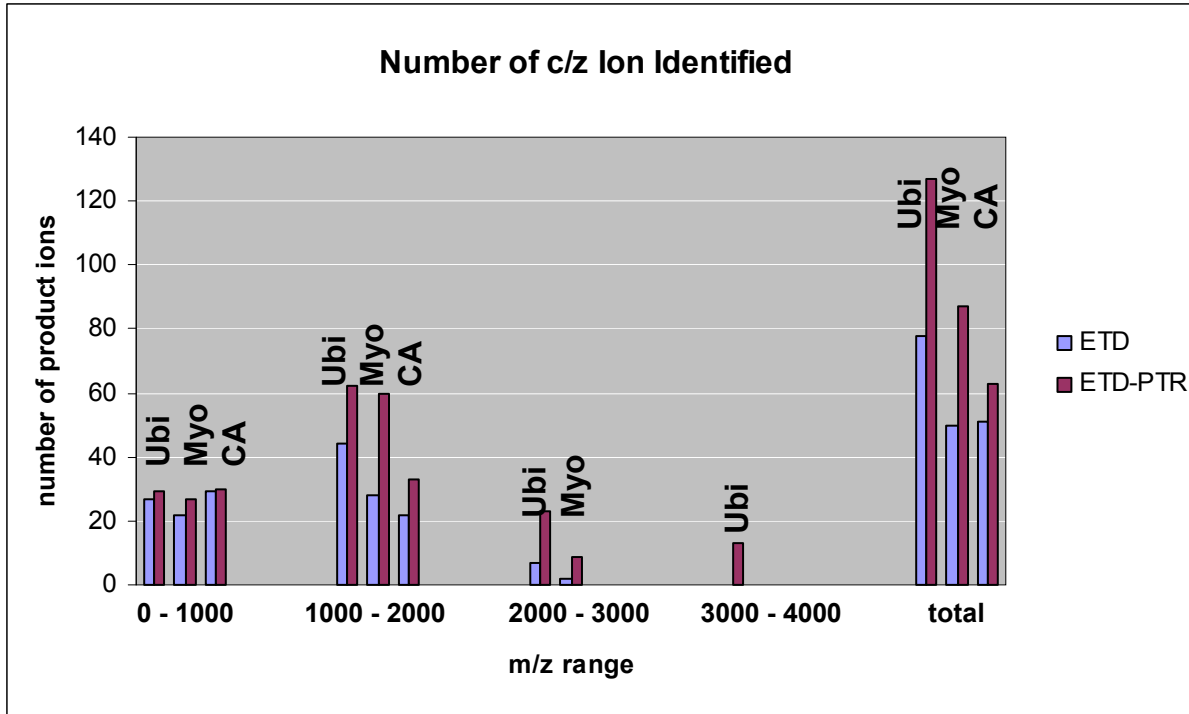


Proton Transfer Reaction (PTR) Simplifies ETD Spectrum.

Ubiquitin (Mw = 8.6 kDa)



LTQ ETD PTR for Top-down – the Limitation



Ubi –ubiquitin, 8 K
Myo – myoglobin, 17 K
CA – Carbonic anhydrase, 29 K

Summary and Conclusion

- ETD is particularly valuable for sequencing intact protein because it randomly cleaves protein backbone bonds, generating information-rich spectra.
- With Orbitrap detection, the complex, well-resolved ETD spectra of intact proteins were easily interpreted. Extensive sequence coverage was obtained from the deconvoluted accurate-mass spectrum.
- Shorter ETD reaction time generated larger product ions and better overall sequence coverage. Extended ETD reaction produced mostly shorter, terminal sequence ions, which is an ideal result for protein N- and C- terminal sequencing.
- However, extended ETD reaction produces c /z ions containing one or more extra hydrogen. Such unexpected mass shift complicates data analysis.

Summary and Conclusion

- PTR following ETD reduces charge carried by product ions, thus the complexity of spectrum.
- By subtracting protons, PTR following ETD generates charge reduced c/z ions that do not contain extra hydrogen. These ions show expected masses for the data analysis software.
- For small intact proteins, LTQ ETD-PTR significantly improves sequence coverage, when compared to ETD (extended reaction) alone. This is true even when the c/z ions containing extra hydrogen from extended ETD are included for ID.
- For larger proteins (>30 K), the benefit of PTR is limited due to the huge number of product ions overlapping with one another, the diluted signal intensity and the limited resolution of LTQ XL. In this case, the sequence ions identified are limited to small, terminal ions.