Putting ECD up front:
Atmospheric Pressure Electron Capture Dissociation

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Faculty of Medicine
University of British Columbia

a place of mind

UppCon '13
Feb. 18, 2013

Vancouver

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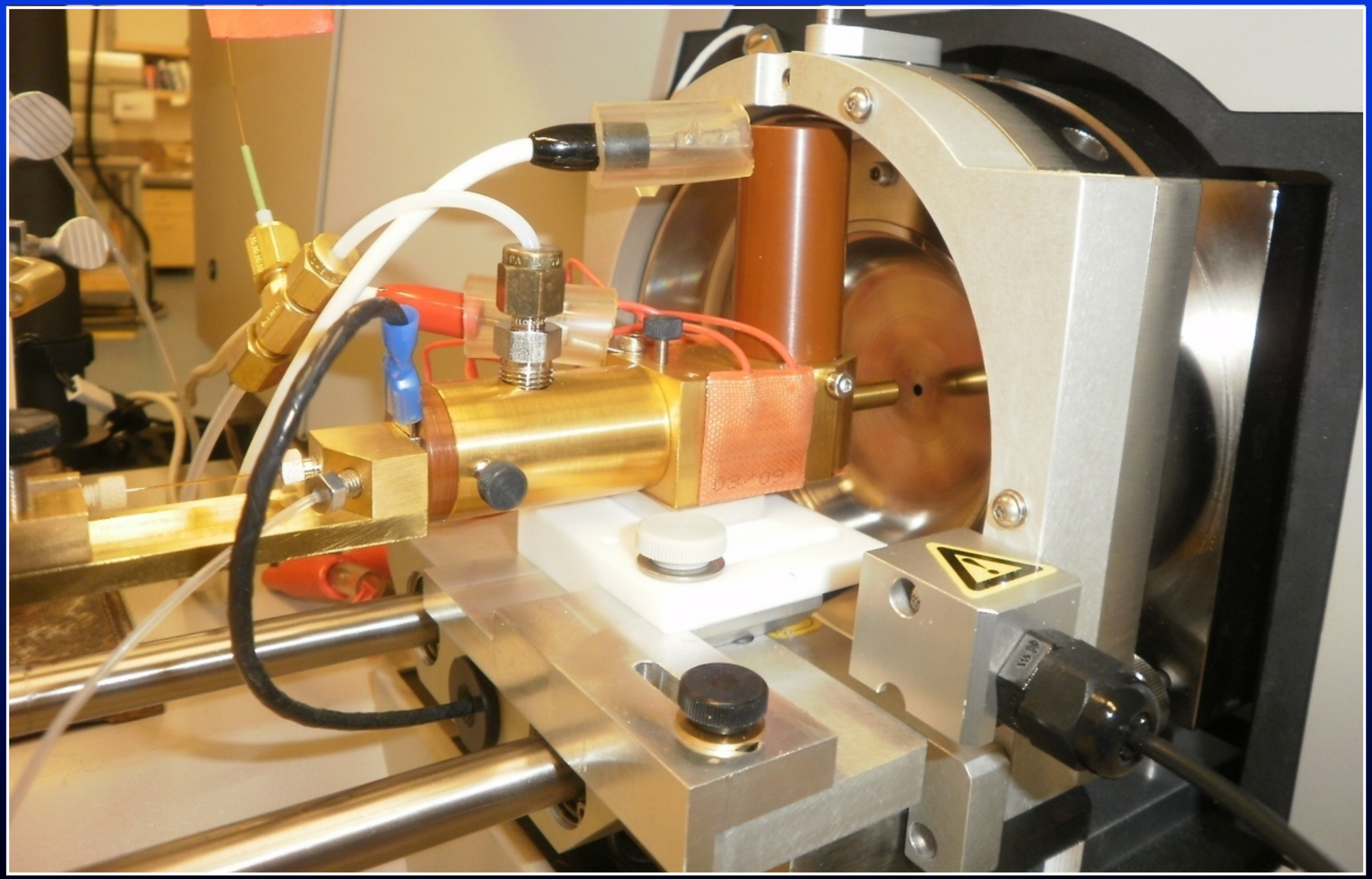
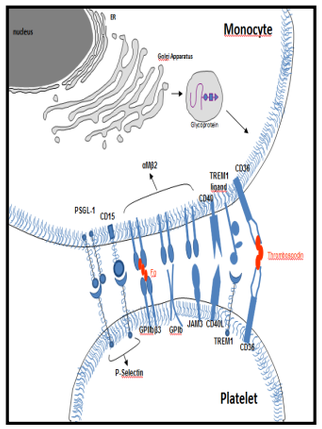
Atmospheric Pressure - Electron Capture Dissociation

Mass Spec hardware design - like a plumber on a small scale

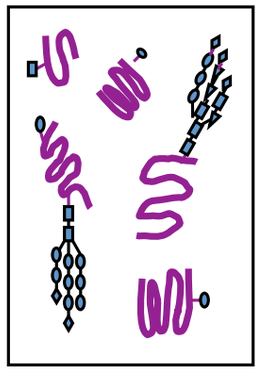


Kast Lab
Where I fit in?

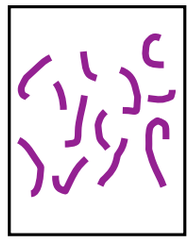


Enrichment

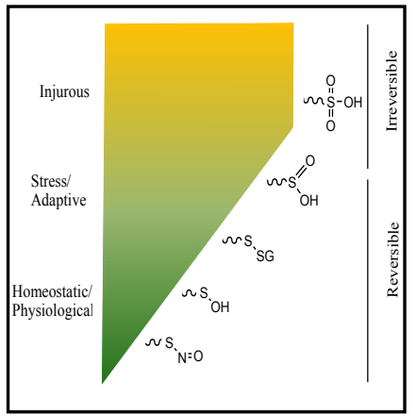
Glycoproteome



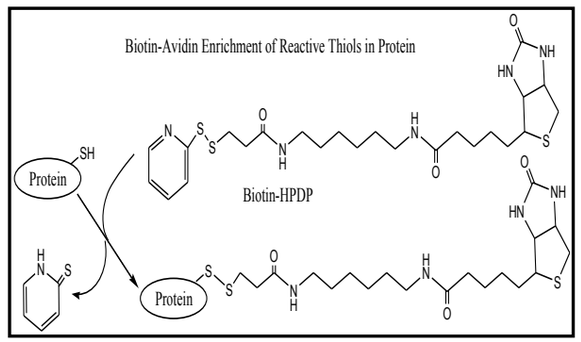
Digestion



glyco modifications



Cell
Lysate

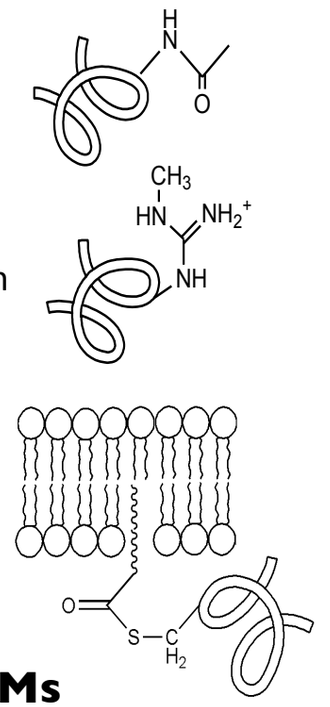


cysteine modifications

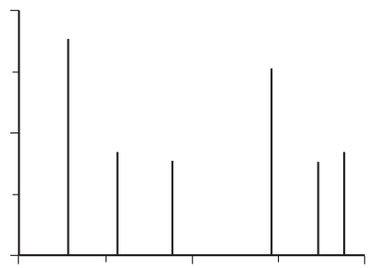
acetylation

methylation

lipidation



other PTMs



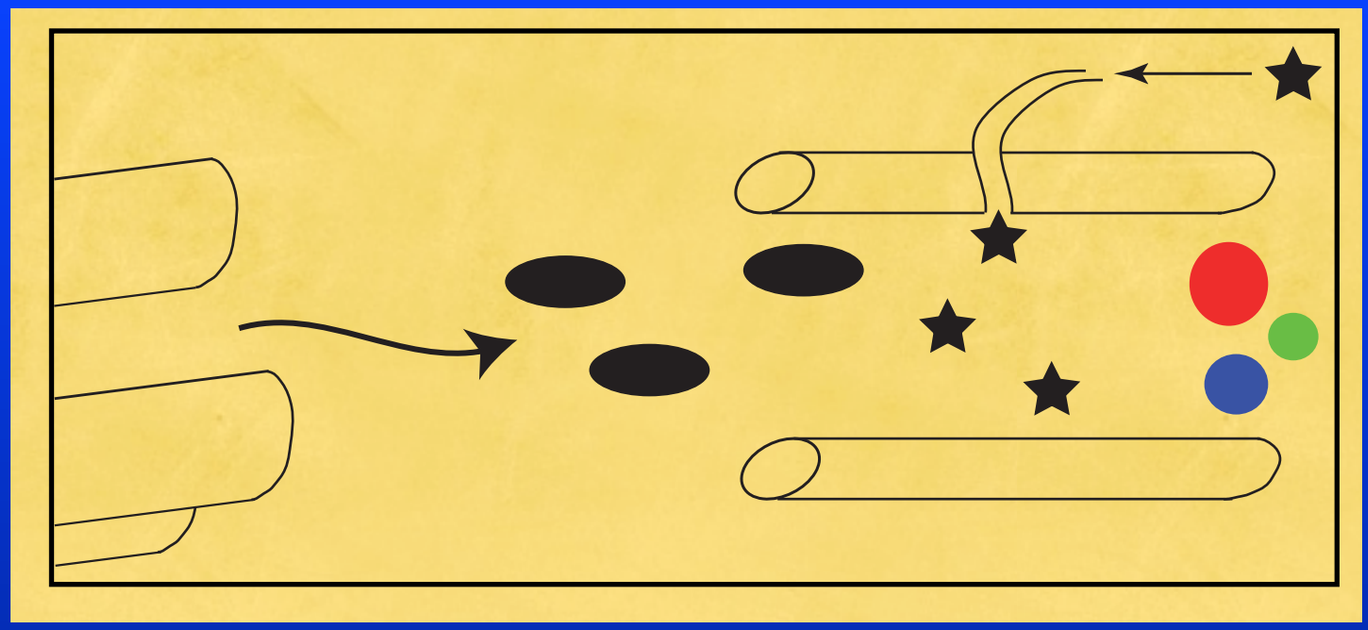
mass spectrometry

Quadrupole Ion Trap

- multiplexed CID
- ECD localization

Quadrupole Time of Flight

Mass Spectrometry
How it is usually done

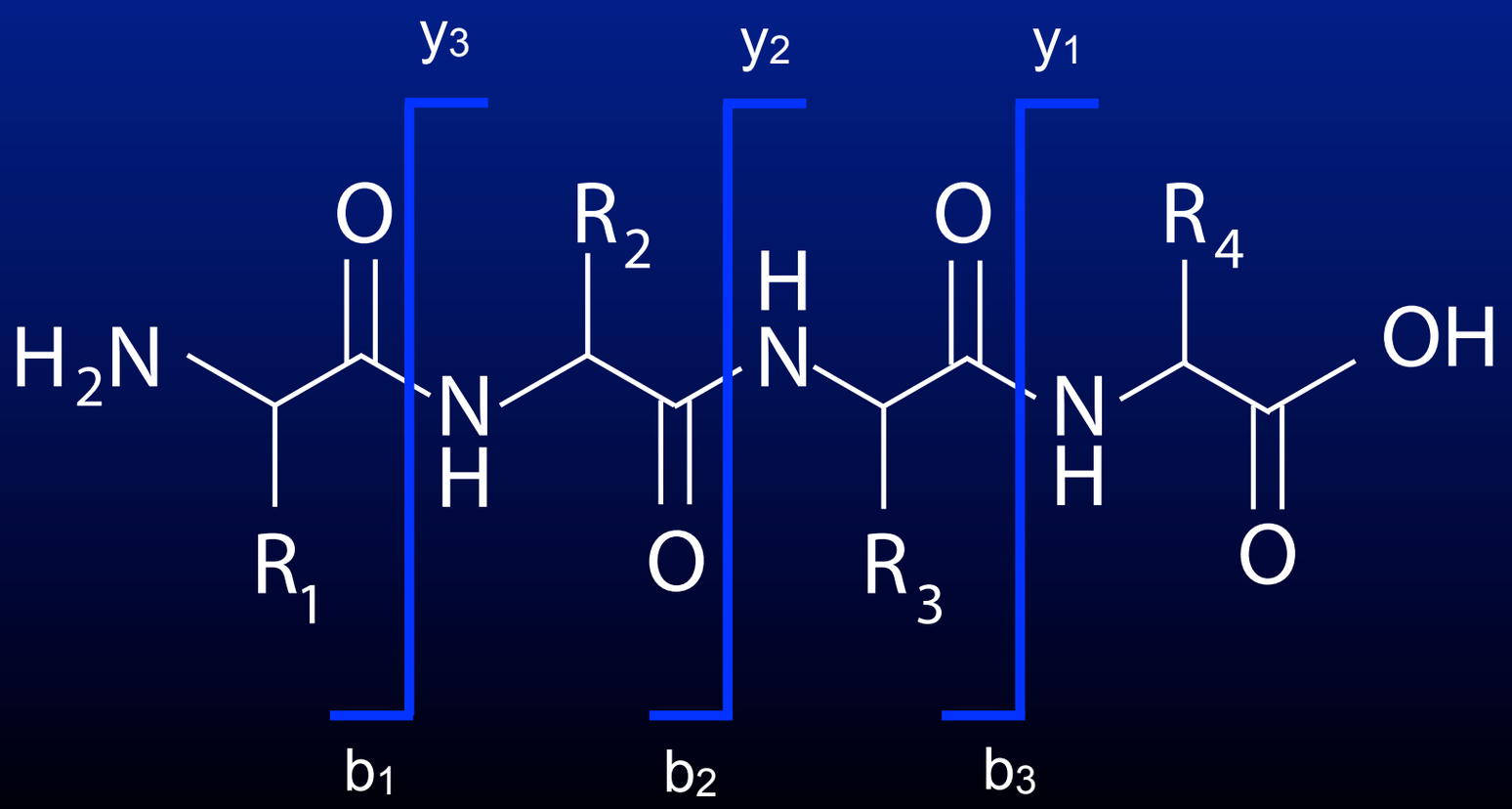


Collision Induced Dissociation

peptides yield b & y ions

not always full sequence coverage

loss of modification, difficult to localize, even PTM scrambling



Atmospheric Pressure - Electron Capture Dissociation

Why?

wanted fuller coverage
wanted to localize labile modifications

Atmospheric Pressure - Electron Capture Dissociation

Why?

Add ECD capacity on our instrument

- fuller coverage
- localize labile modifications
- adapt to our existing workflows/ instrument

Q ToF (2002)

Atmospheric Pressure - Electron Capture Dissociation
First Report

Anal. Chem. **2003,** *75,* 5961−5968

Characterization of Hydrophobic Peptides by Atmospheric Pressure Photoionization-Mass Spectrometry and Tandem Mass Spectrometry

Arnaud Delobel,[†] Frédéric Halgand,[†] Barbara Laffranchise-Gosse,[†] Henri Snijders,[‡] and Olivier Laprévote*,[†]

unexpected c ions from PhotoSpray source at atmospheric pressure
side product of Atmospheric Pressure PhotoIonization

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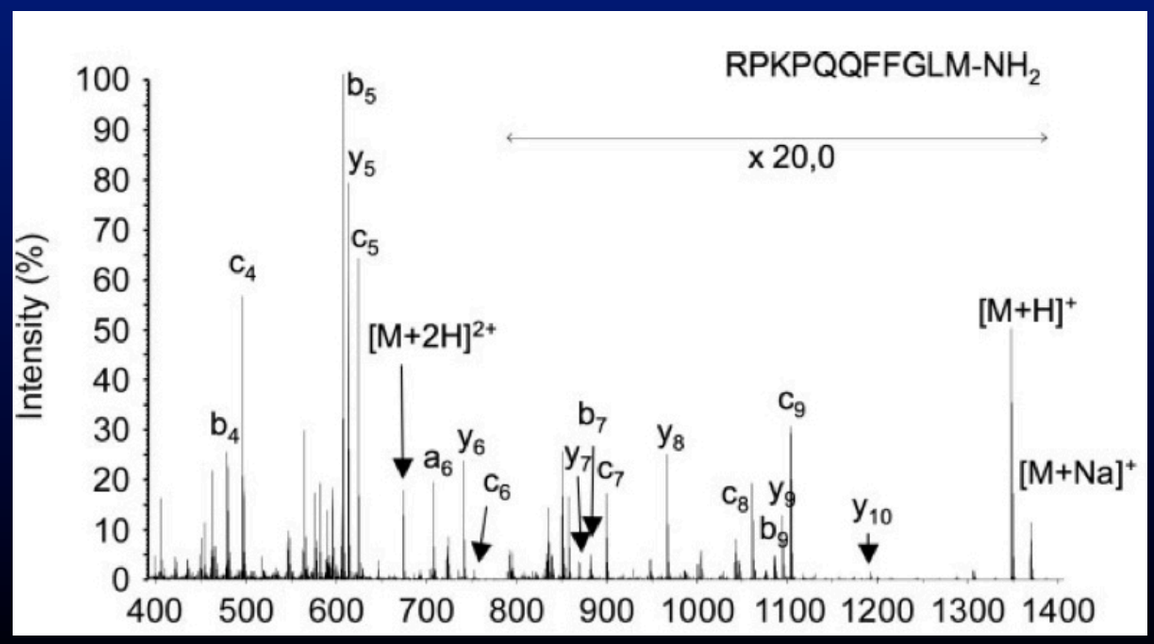
unexpected c ions from PhotoSpray source at atmospheric pressure side product of Atmospheric Pressure PhotoIonization

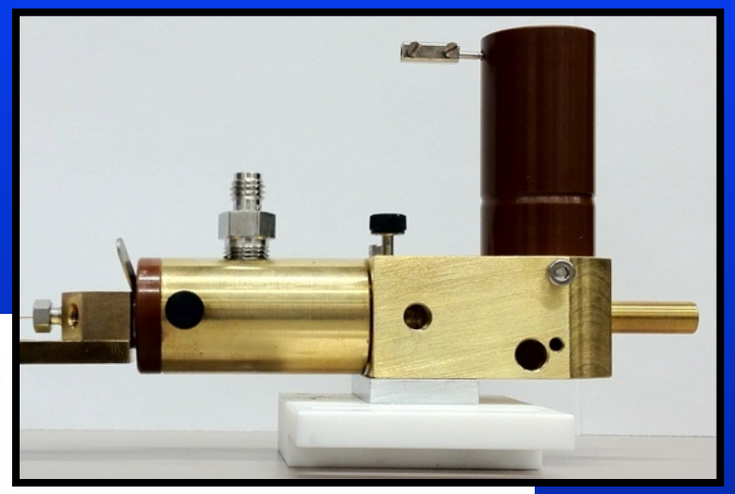
required large concentrations

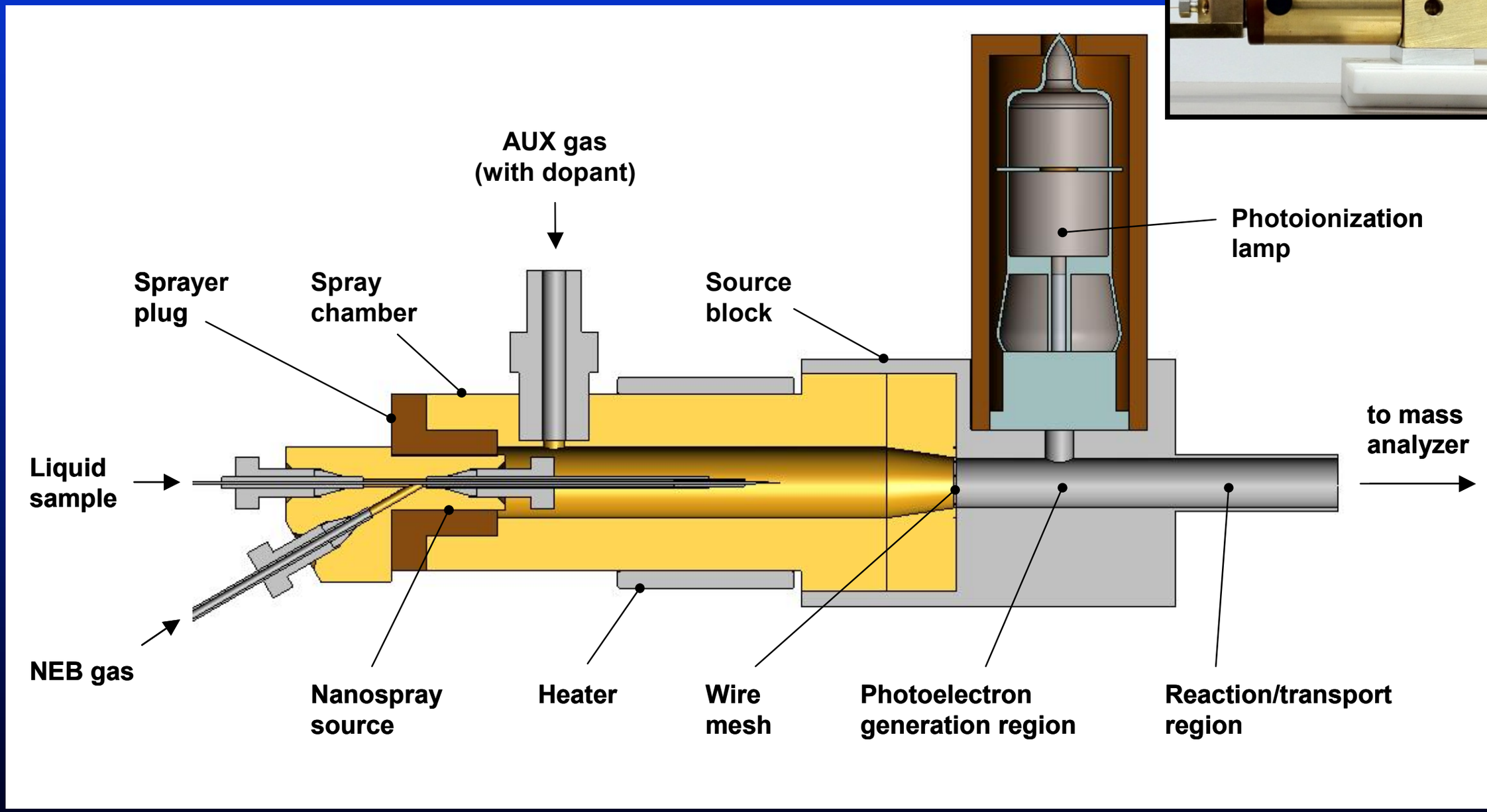


Debois et al., 2006, J. Mass Spectrom

Atmospheric Pressure - Electron Capture Dissociation

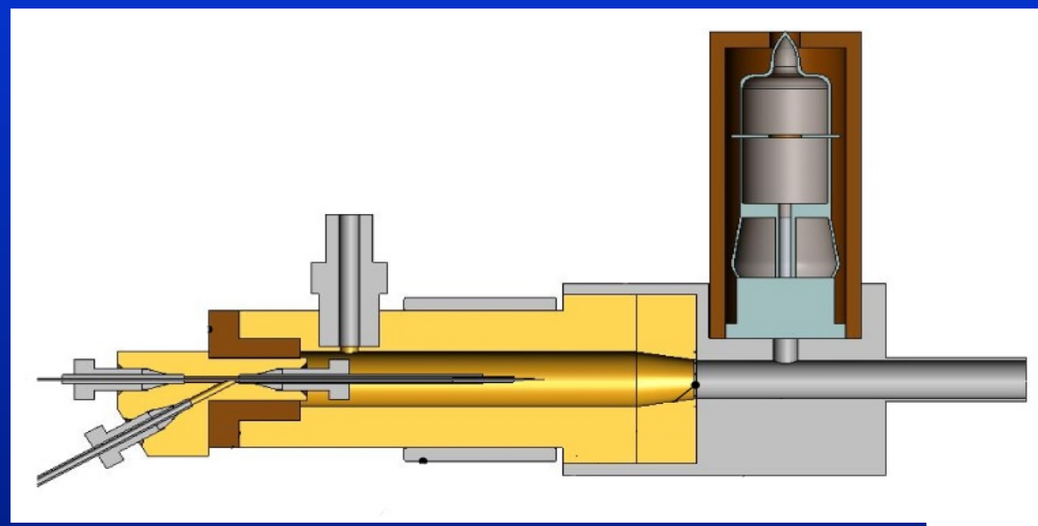
How it works?

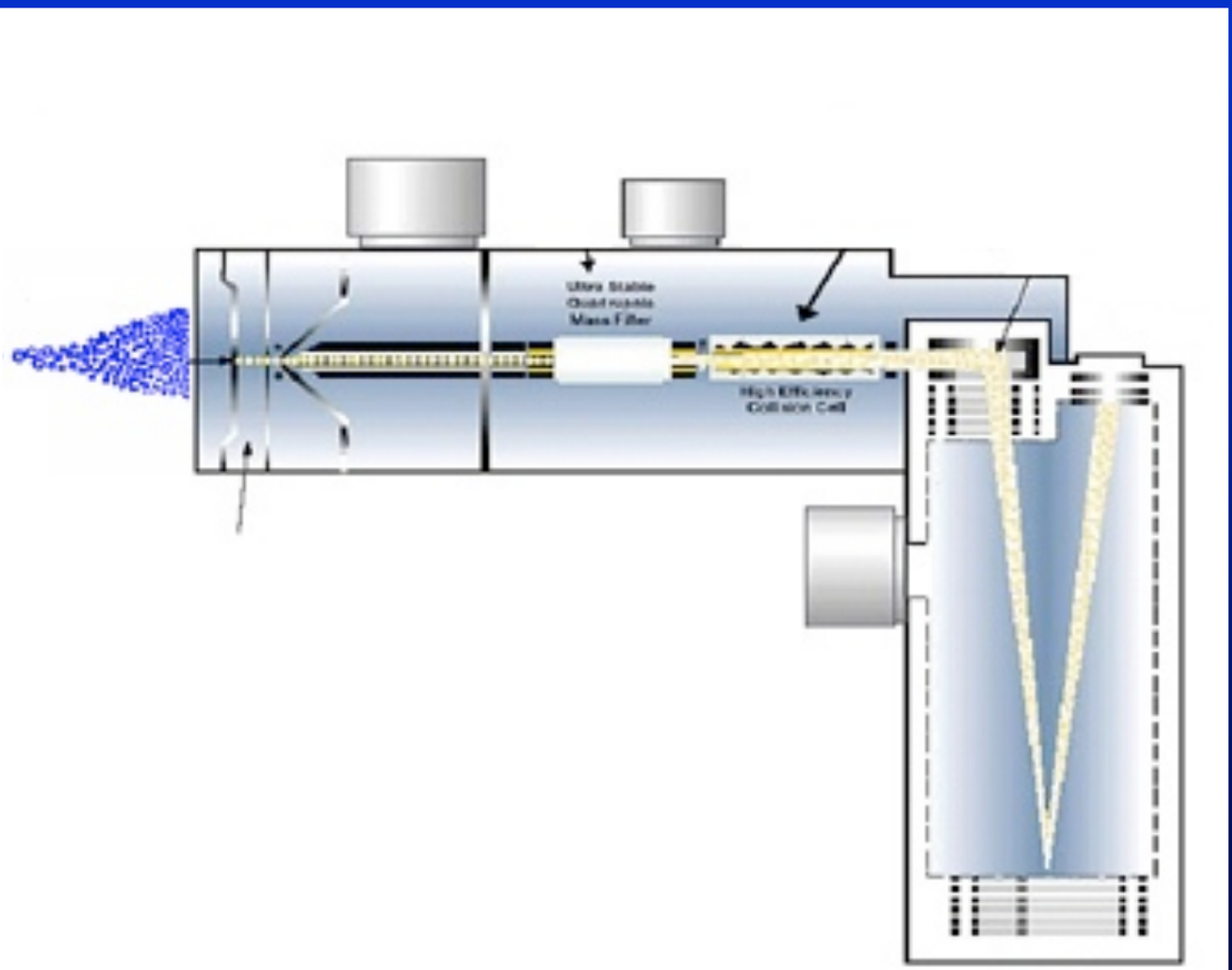




$$\text{acetone}\ \text{IE 9.7 eV} + h\nu\ 10\ \text{eV} \rightarrow \text{acetone}^{+} + e^{-}\ \text{KE=0.3 eV}$$

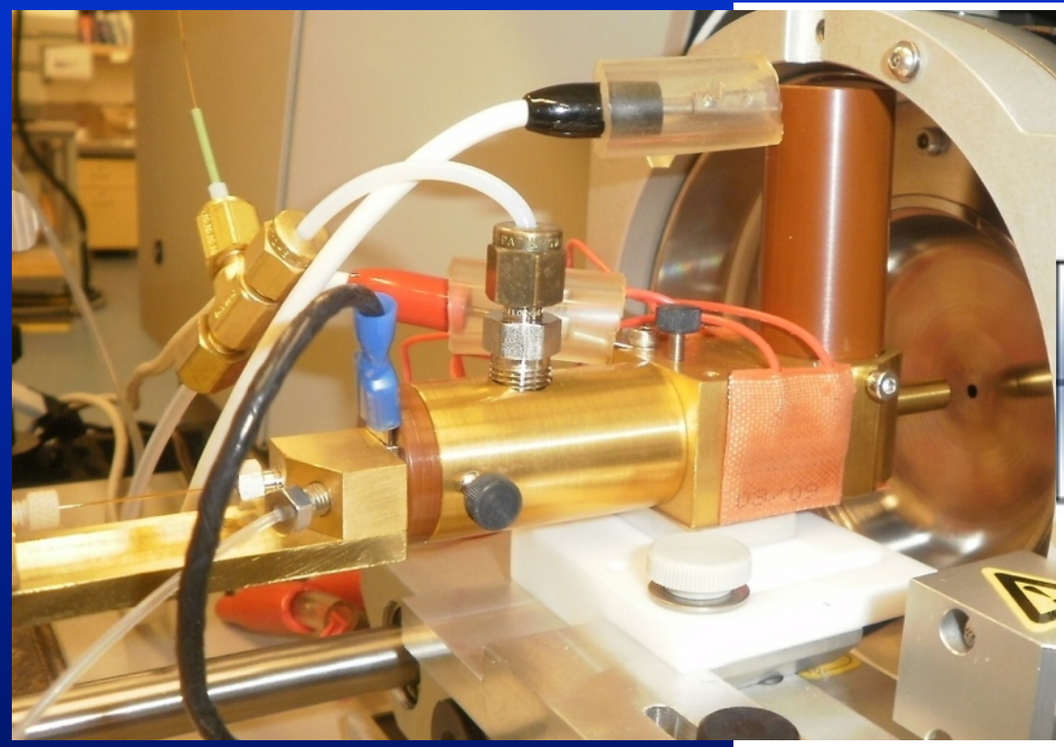
Atmospheric Pressure - Electron Capture Dissociation
Where is goes

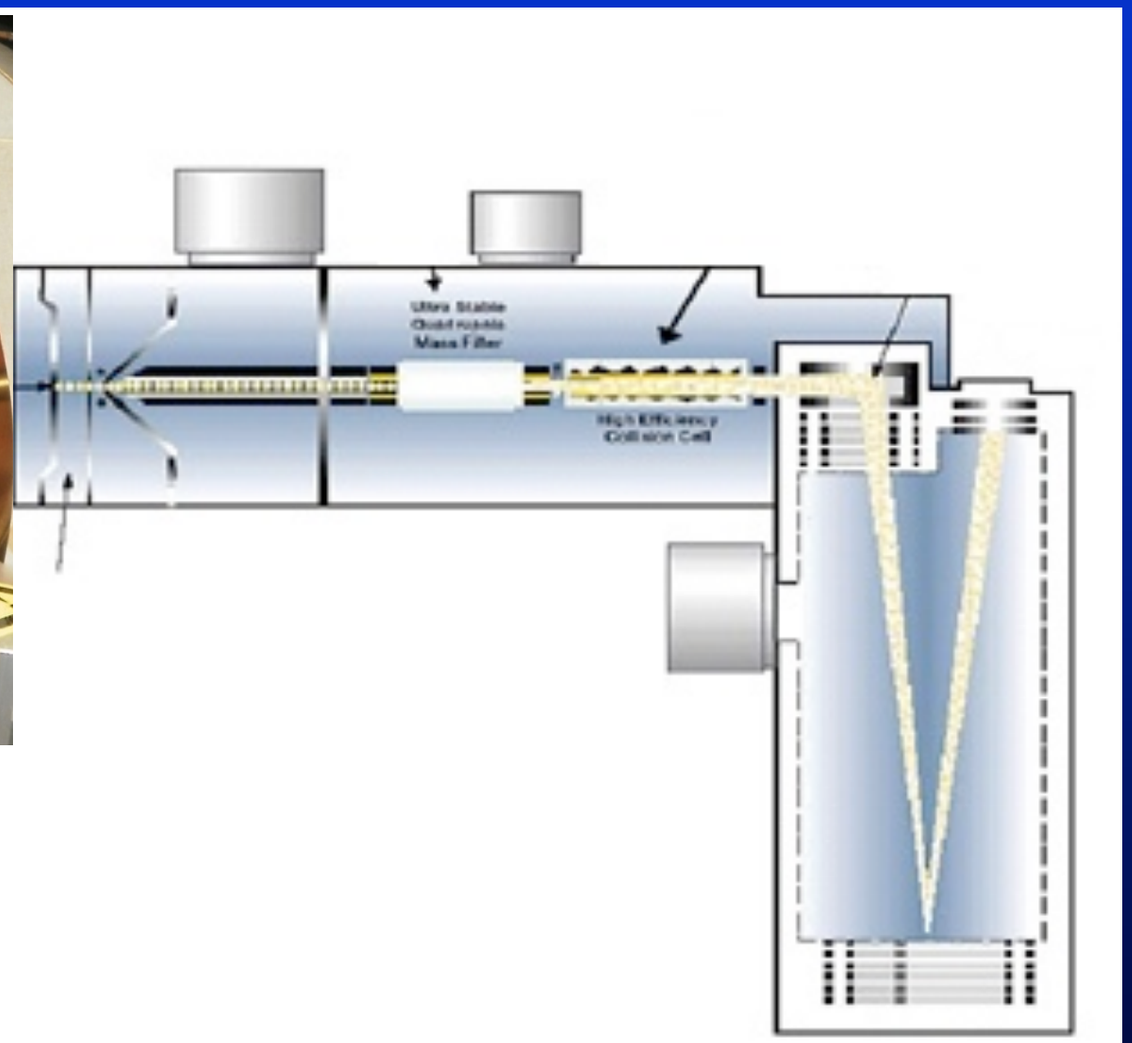




nESI AP-ECD Q CID TOF

Atmospheric Pressure - Electron Capture Dissociation
Where is goes

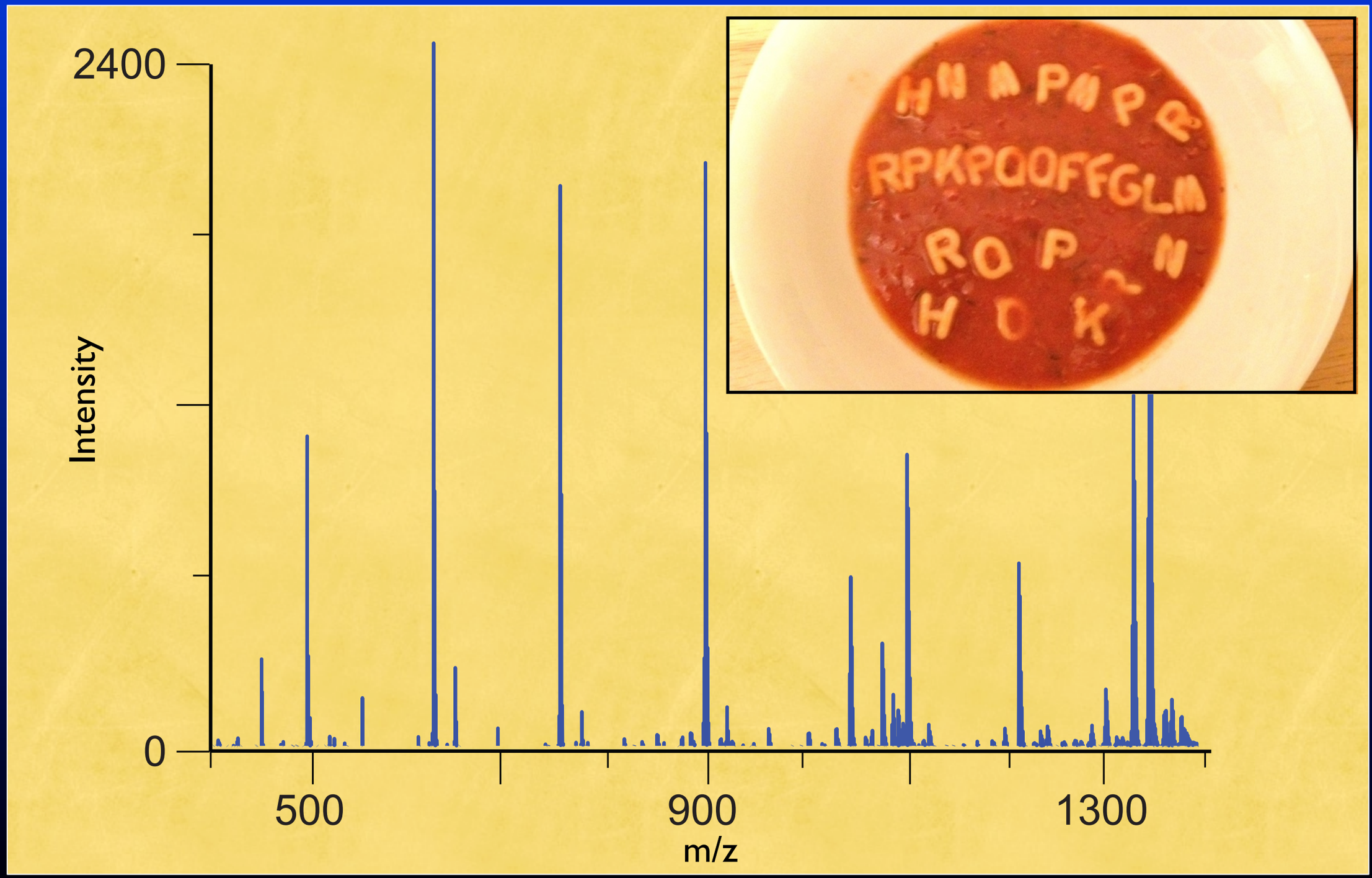




nESI AP-ECD Q CID TOF

Atmospheric Pressure - Electron Capture Dissociation
Proof of Concept

ECD fragmentation of infused Substance P (no modifications)



Robb et al., JASMS, 2011
Robb et al., RCM, 2010

Atmospheric Pressure - Electron Capture Dissociation
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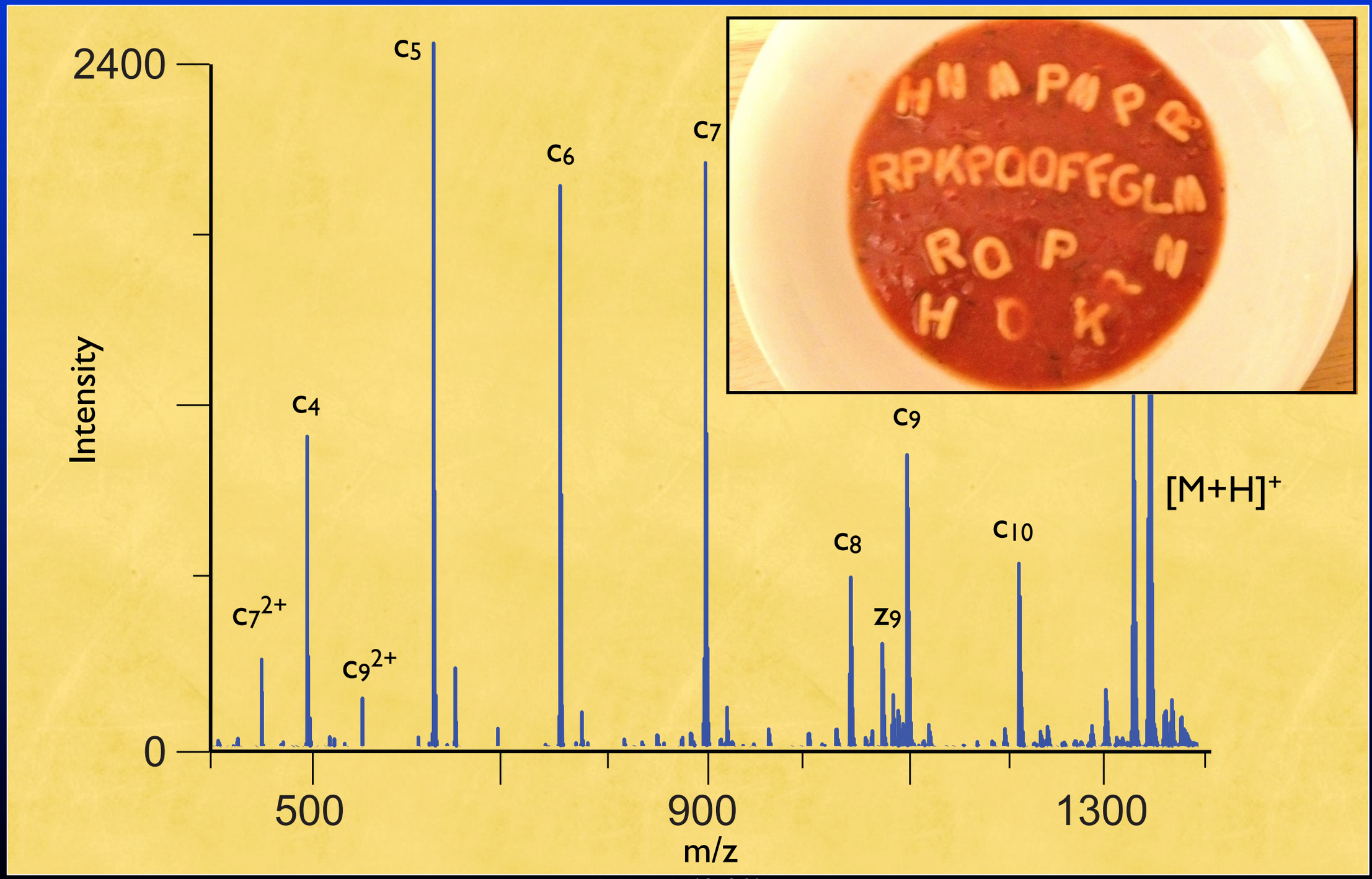

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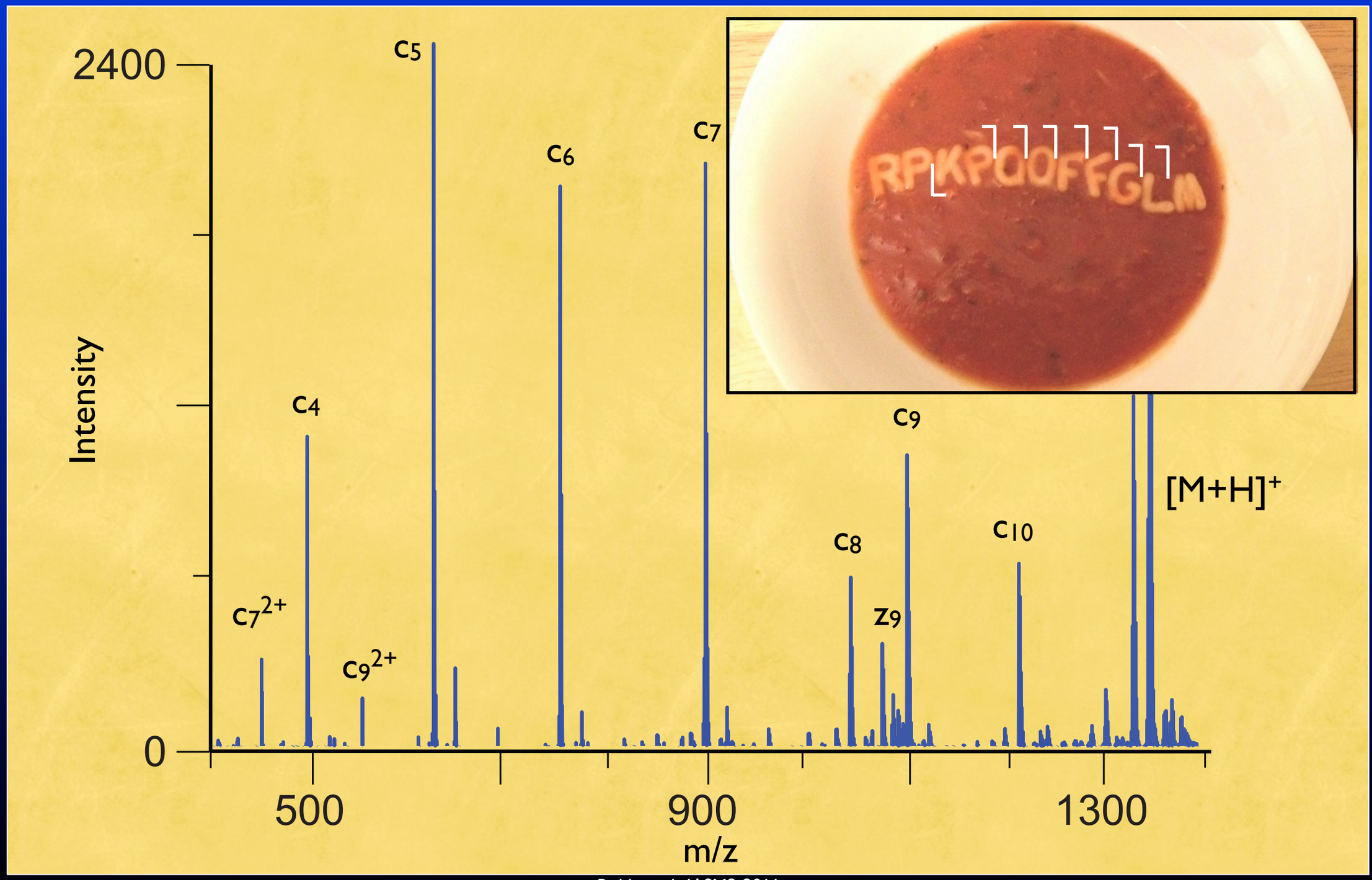
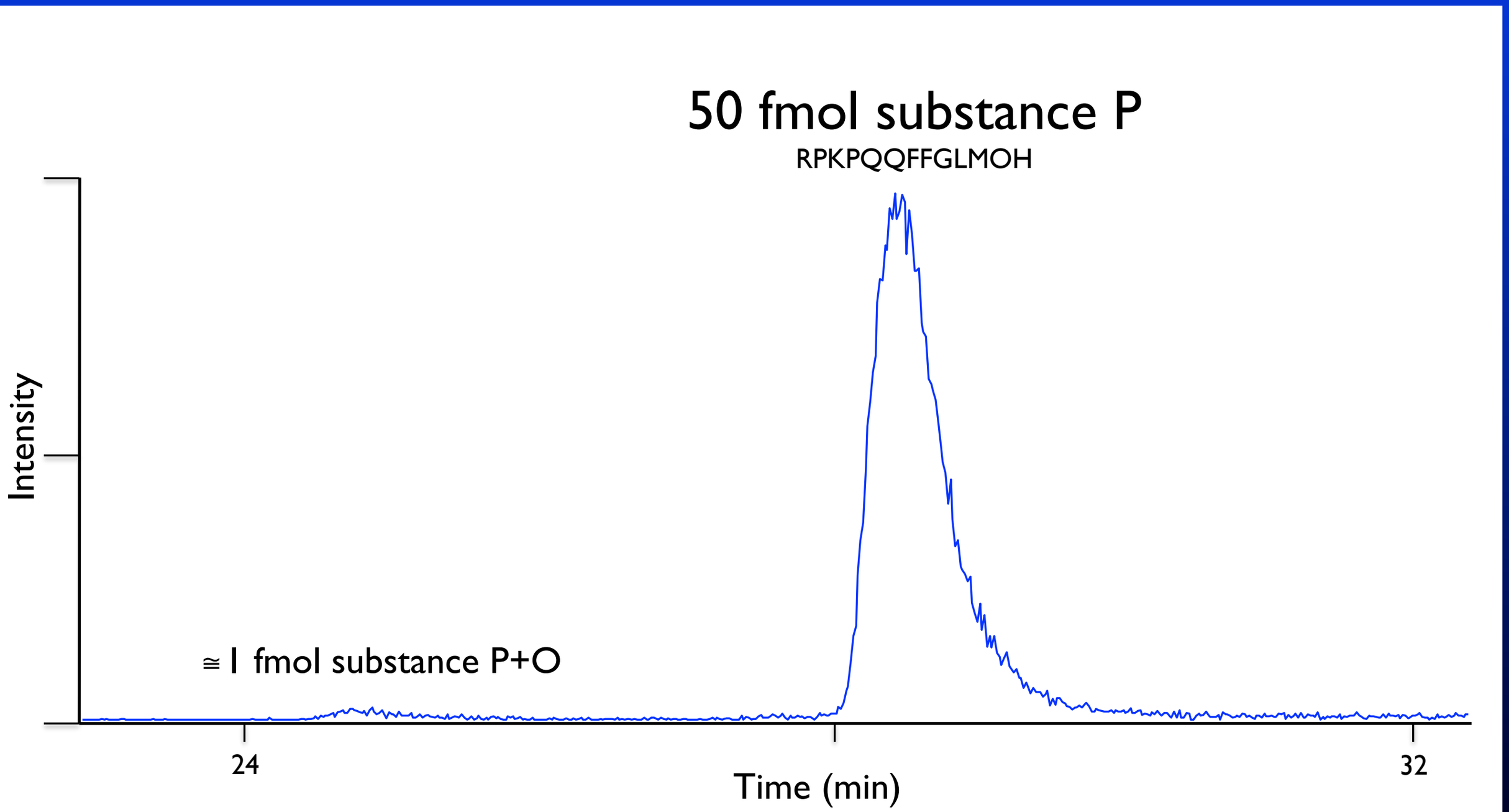


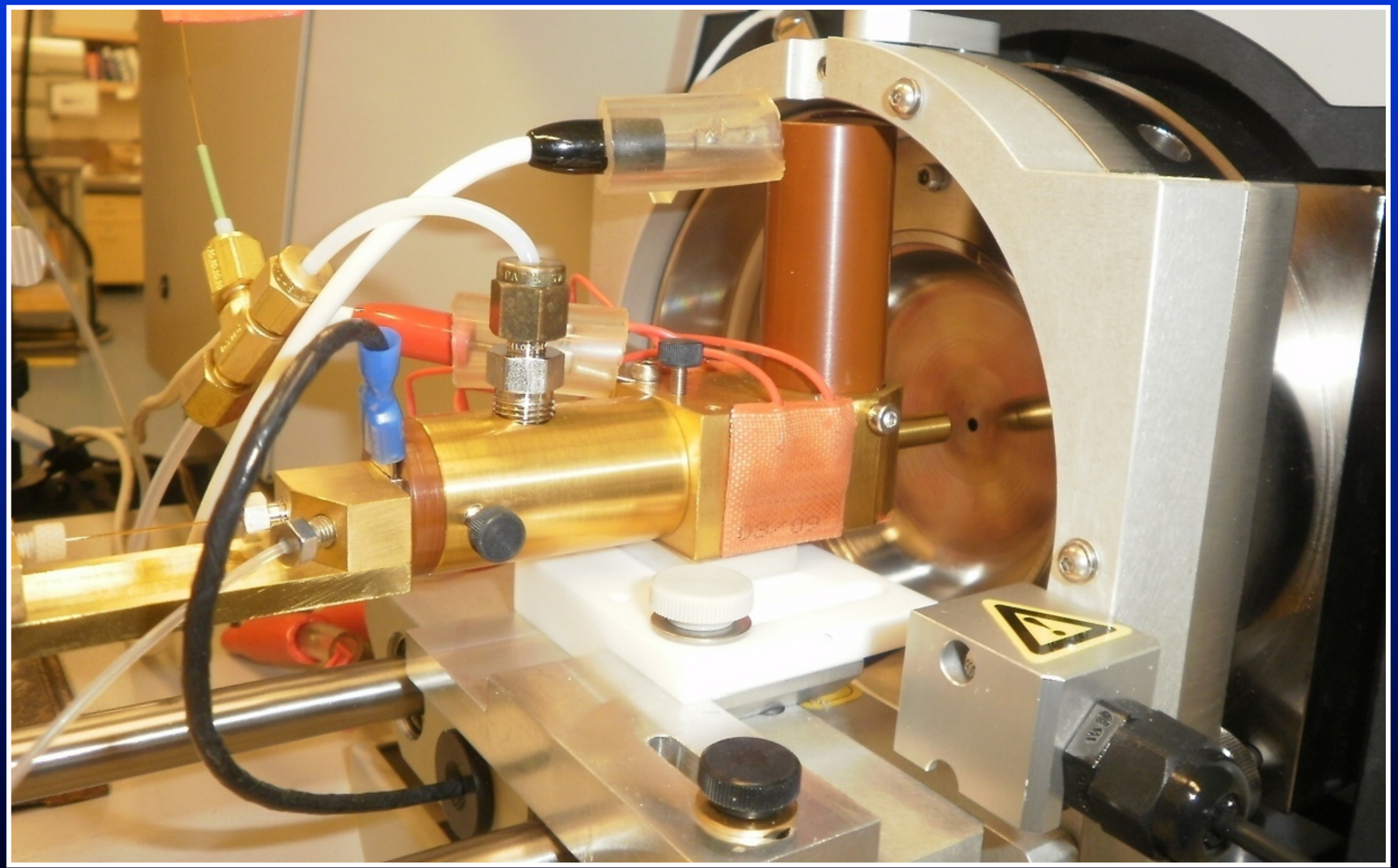
Robb et al., JASMS, 2011
Robb et al., RCM, 2010

Evaluation of AP - ECD
Sensitivity



Robb et al., Anal. Chem., 2012

Atmospheric Pressure - Electron Capture Dissociation
Where is goes

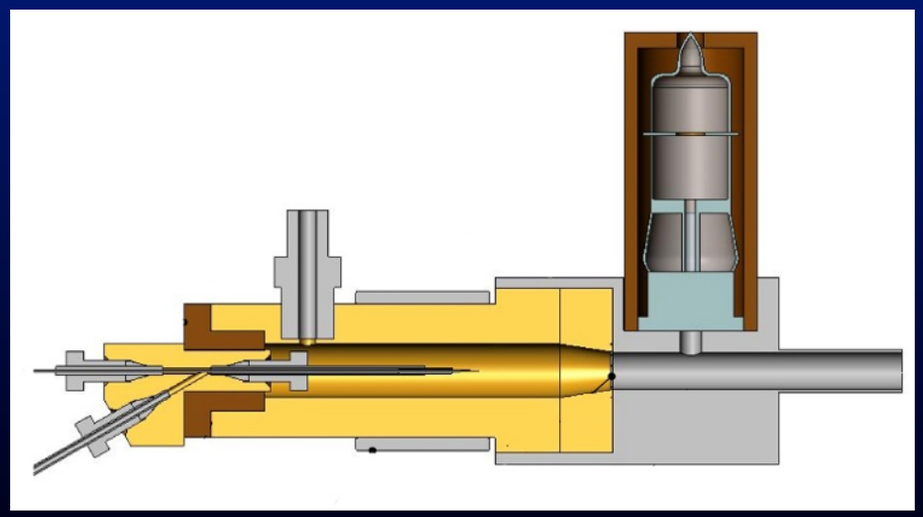


Evaluation of AP - ECD
Reproducibility

Problem: Unknown PM (performance/ maintenance) schedule

Test: run it till it breaks

Result: ran continuously for 8 days (150 injections) with no loss of signal
stopped experiment early (could spend a year testing it)



Evaluation of AP - ECD
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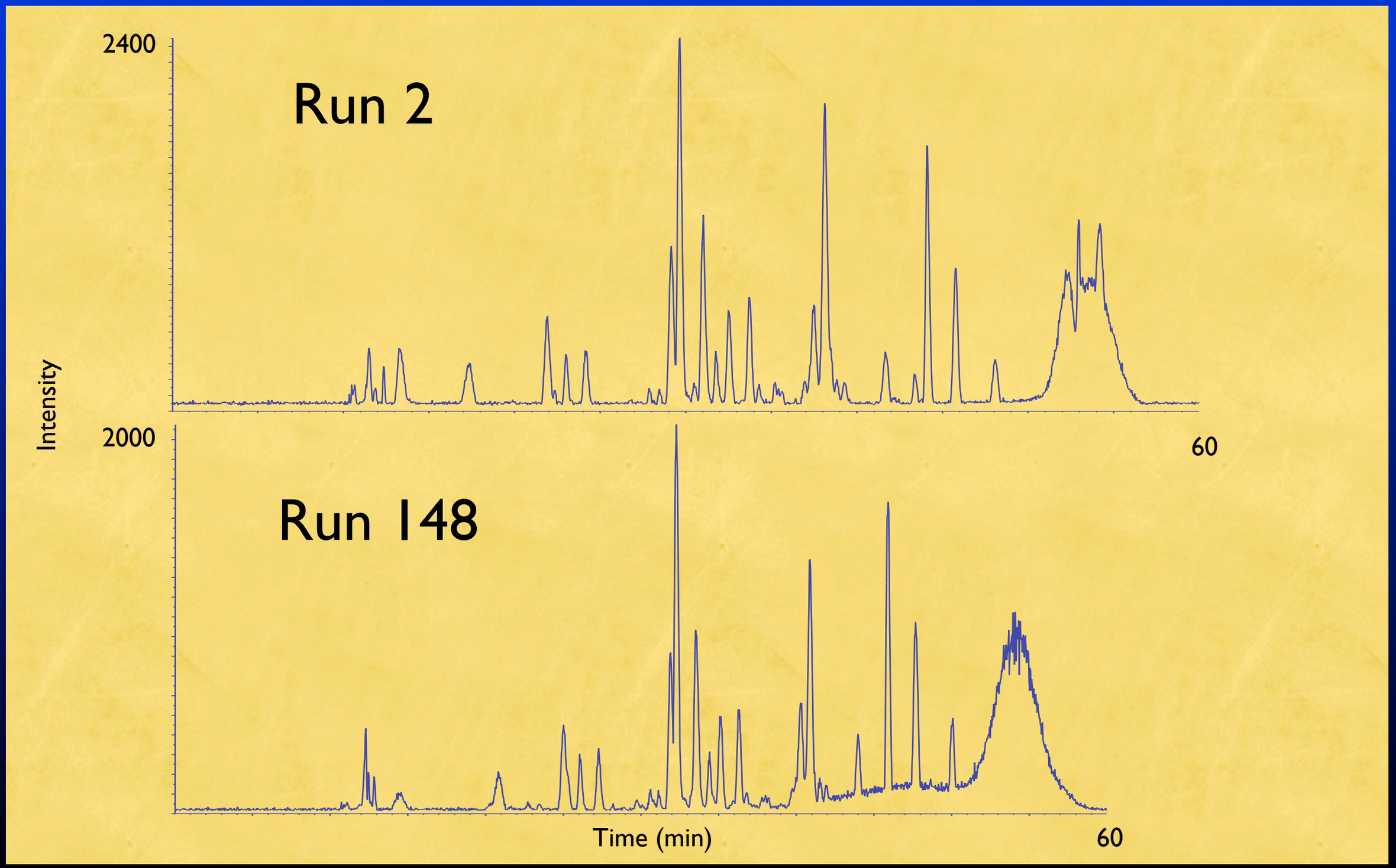

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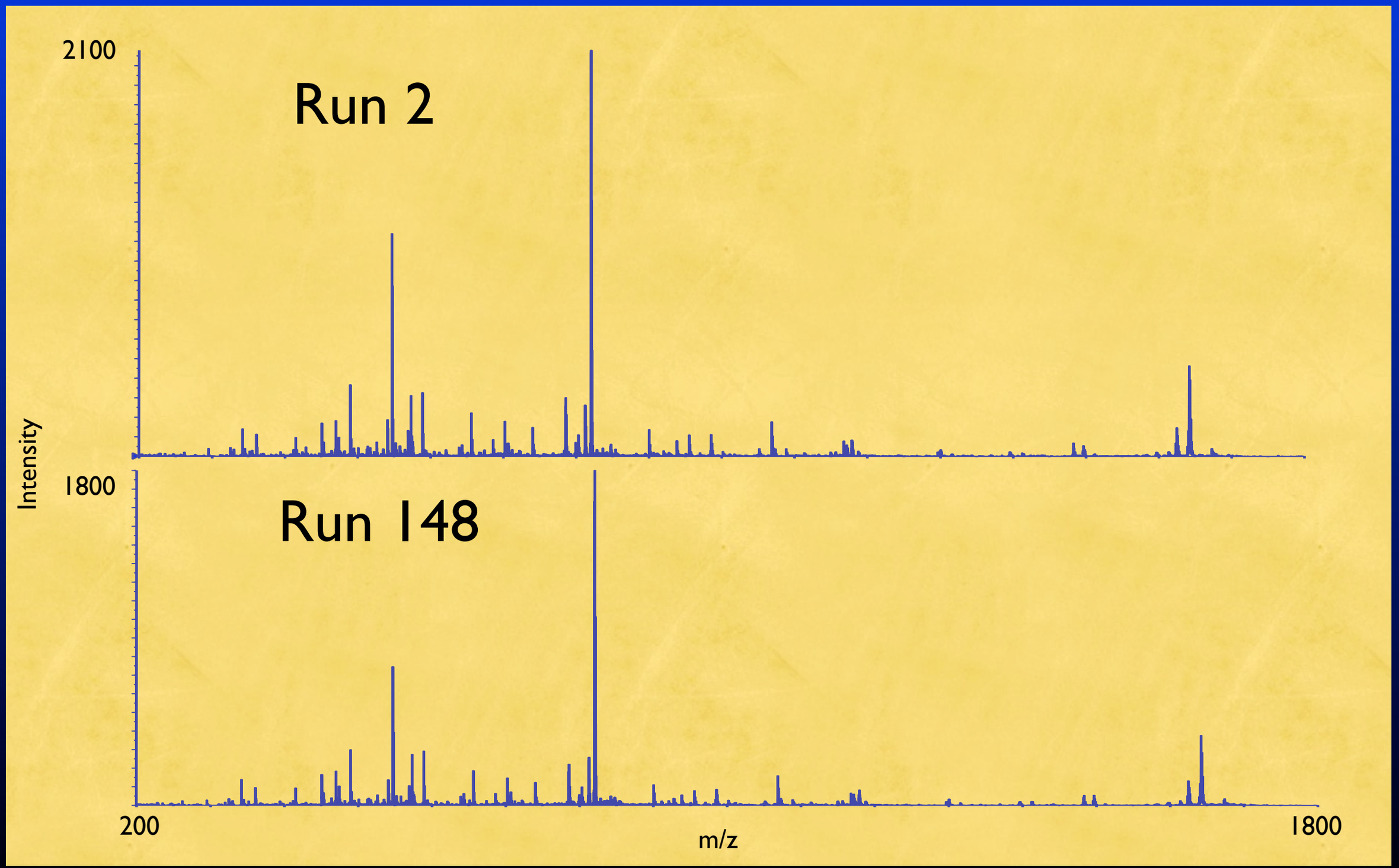
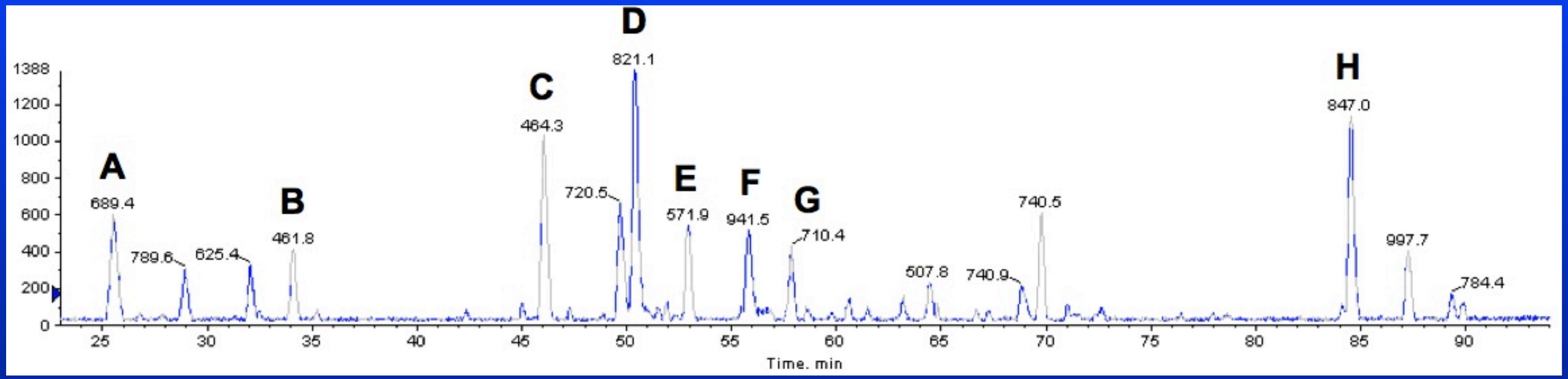
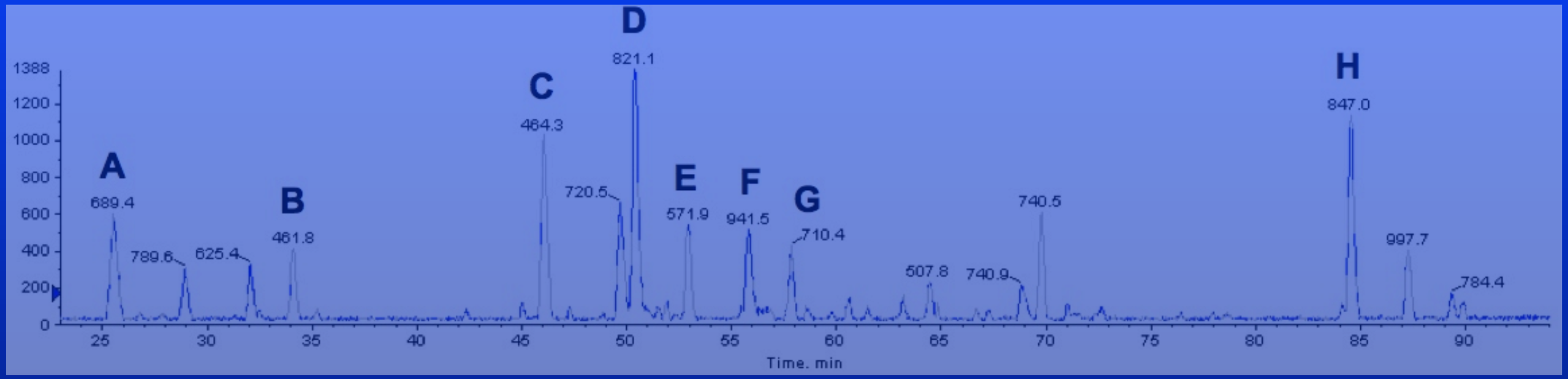


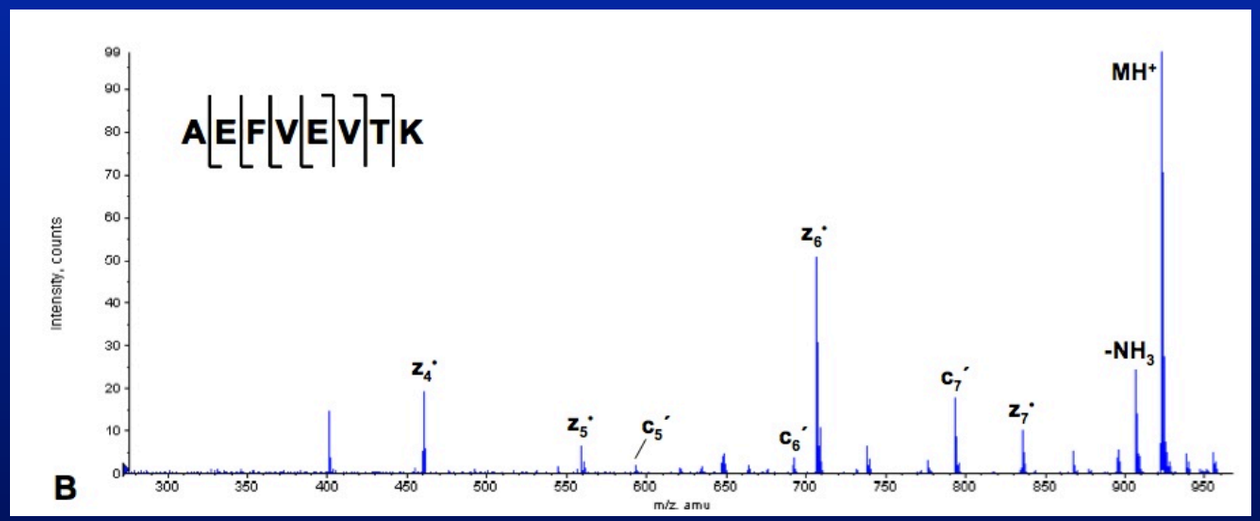
Evaluation of AP - ECD
Mixtures

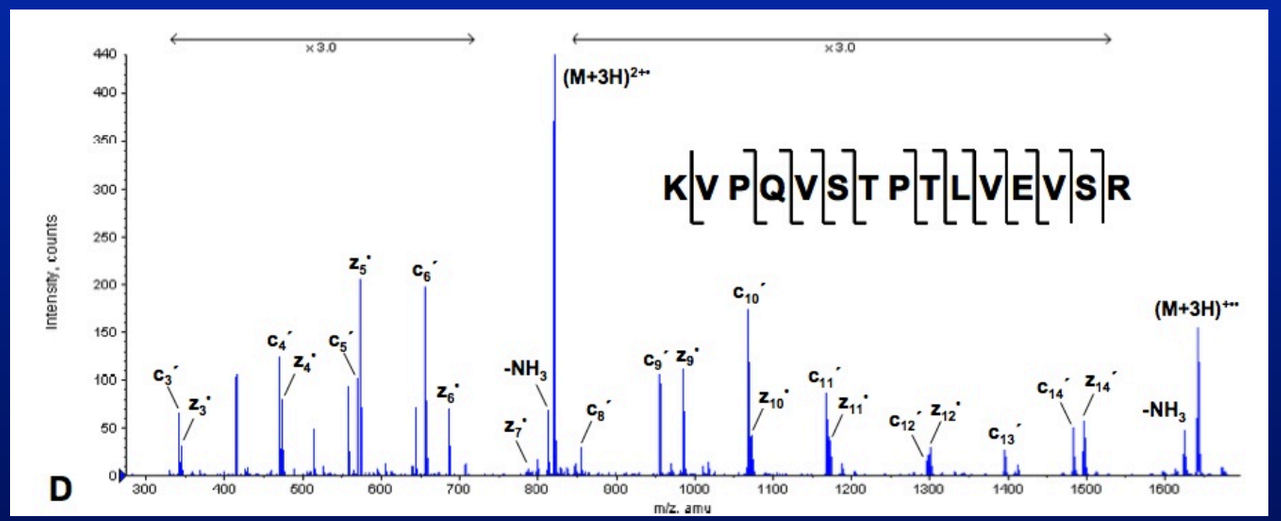


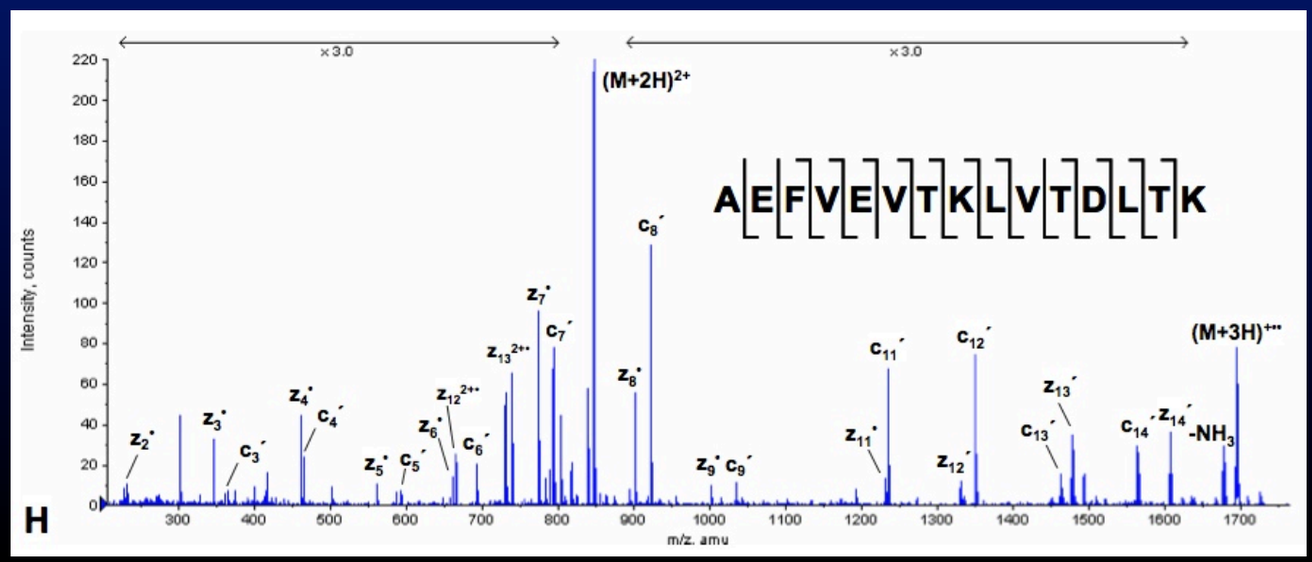
Robb et al., Anal. Chem., 2012

Evaluation of AP - ECD
Mixtures









Robb et al., Anal. Chem., 2012

Evaluation of AP - ECD

AP-ECD Checklist

✓ Reproducible

✓ Sensitive

✓ Peptide mixtures

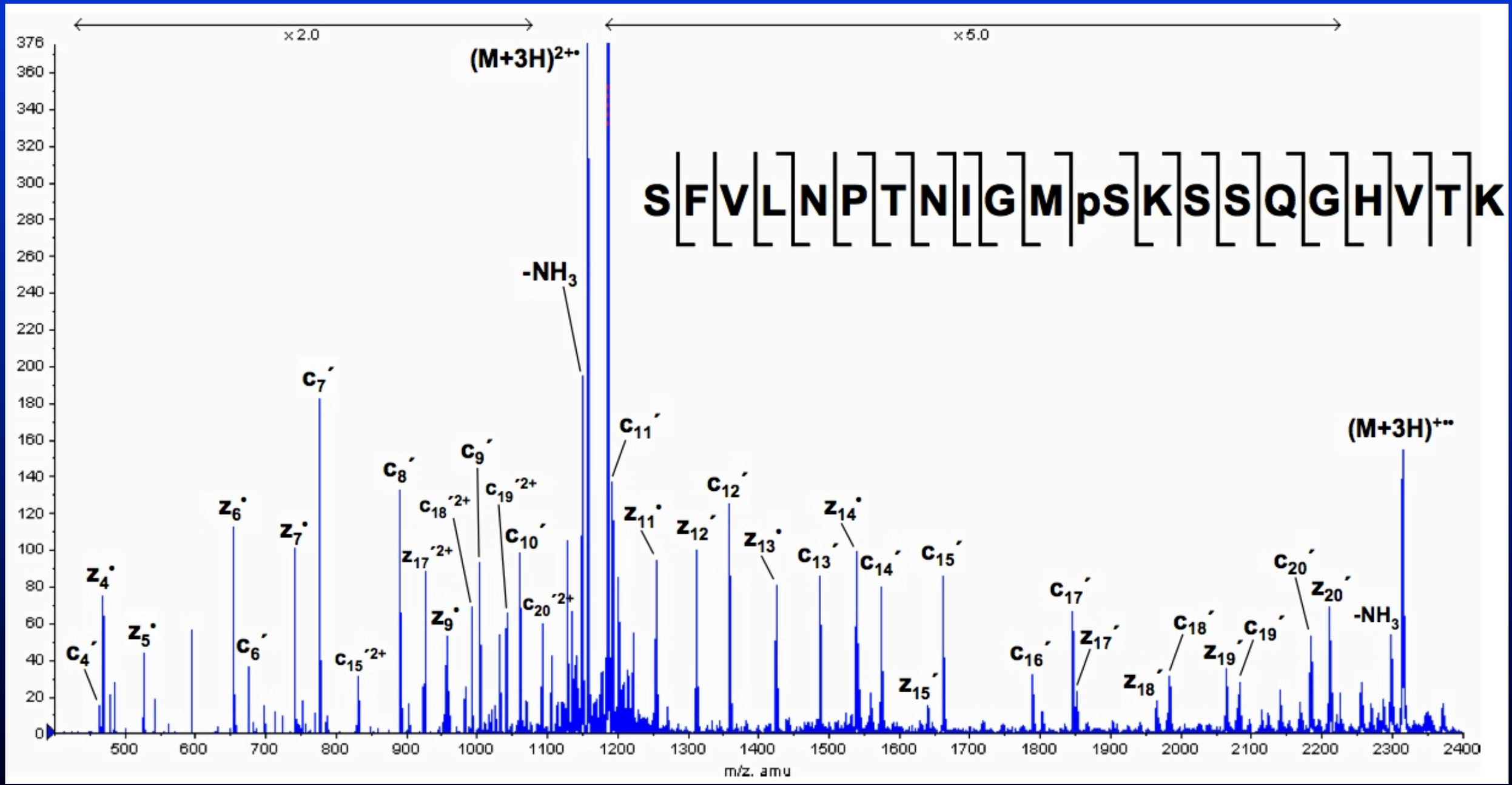
Labile Modifications

glycopeptide

phosphopeptide

sulfopeptide

Evaluation of AP - ECD
Labile modifications #1: Phosphopeptides

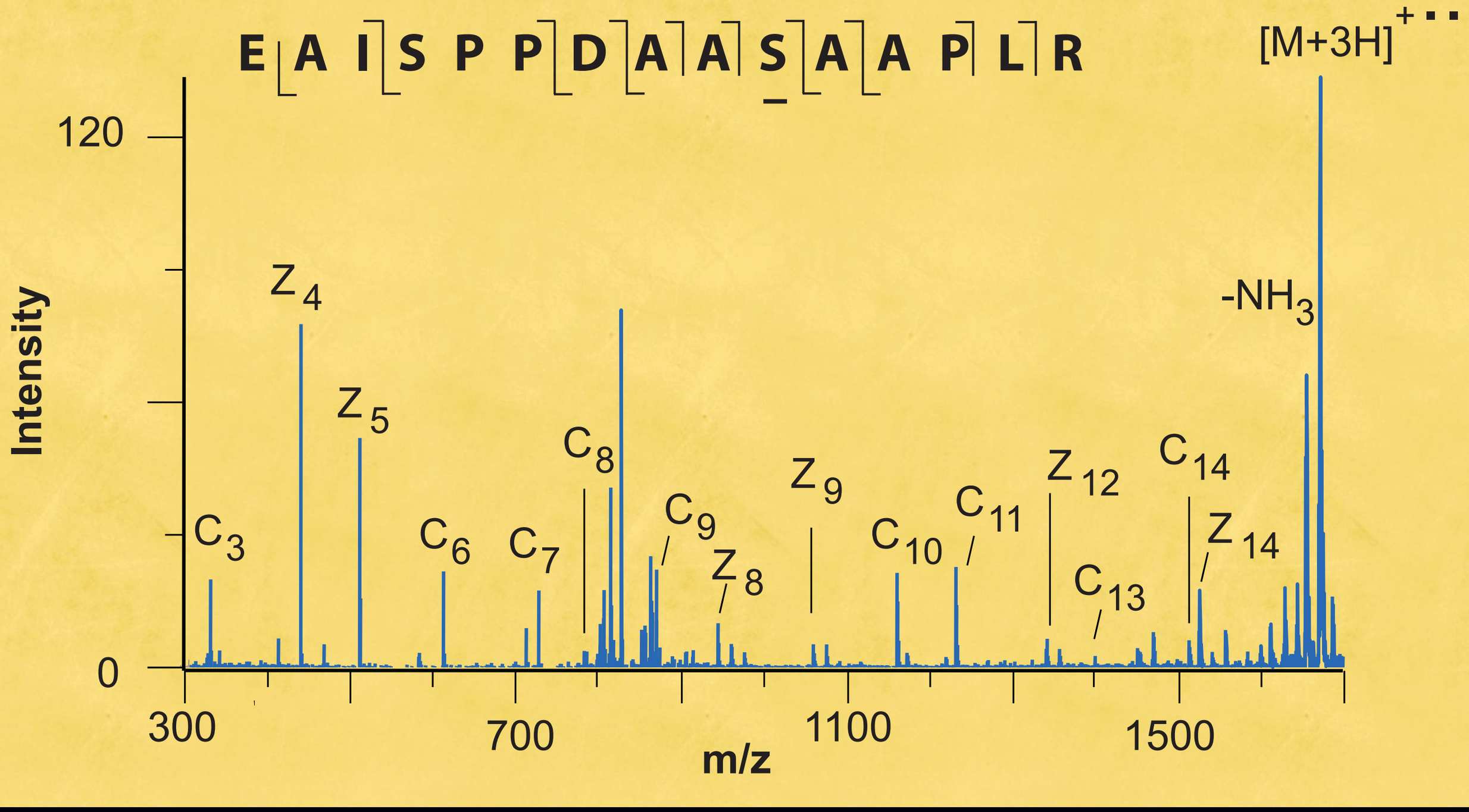


Robb et al., Anal. Chem., 2012

Evaluation of AP - ECD
Labile modifications #2: Glycosylation

E A I S P P D A A S A A P L R

Evaluation of AP - ECD
Labile modifications #2: Glycosylation

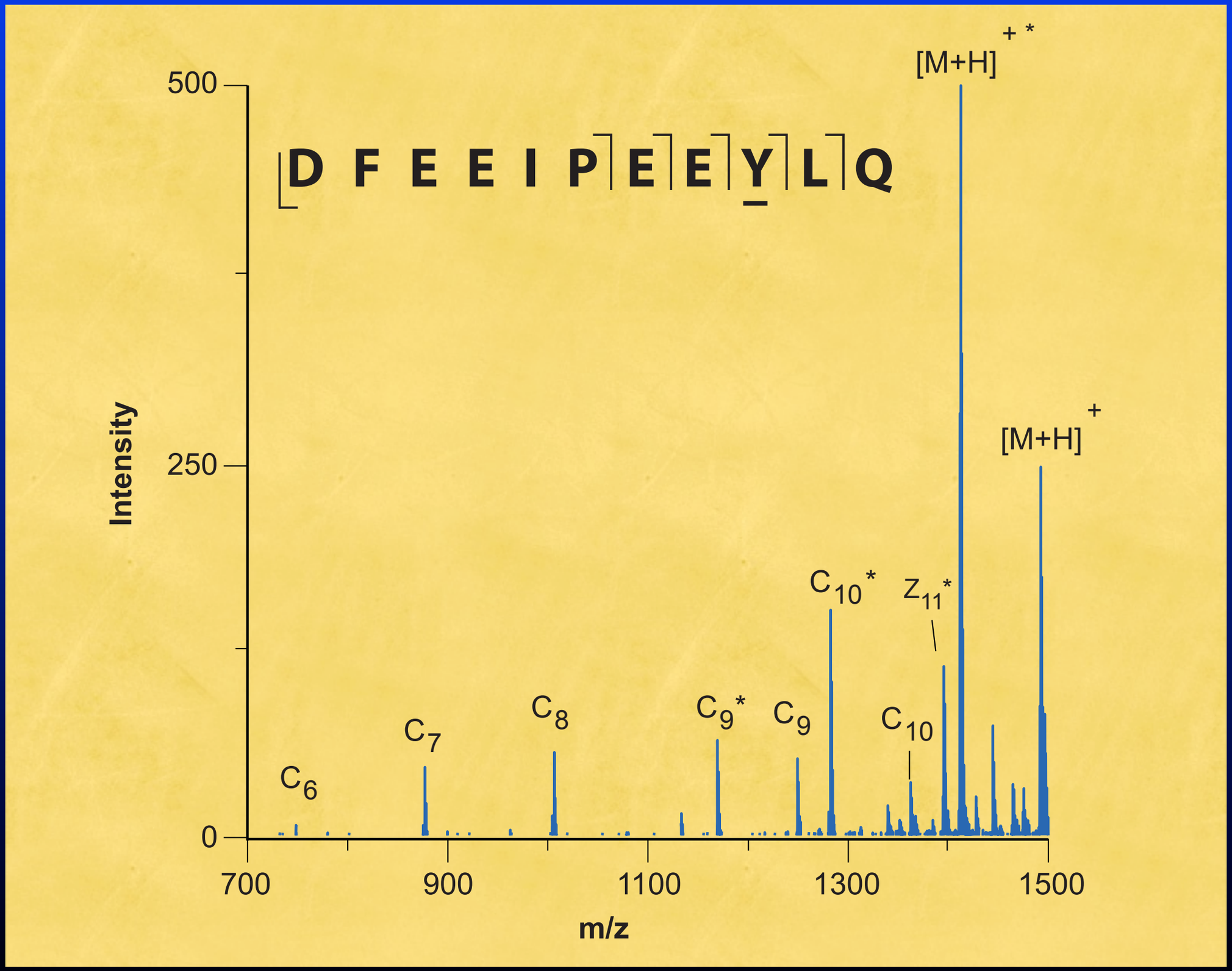


Evaluation of AP - ECD
Labile modifications #3: Sulfopeptide Hirudin

D F E E I P E E Y L Q

Evaluation of AP - ECD

Labile modifications #3: Sulfopeptide Hirudin



Evaluation of AP - ECD
Labile modifications #3: Sulfopeptide Caerulein

Q Q D Y T G W M D F

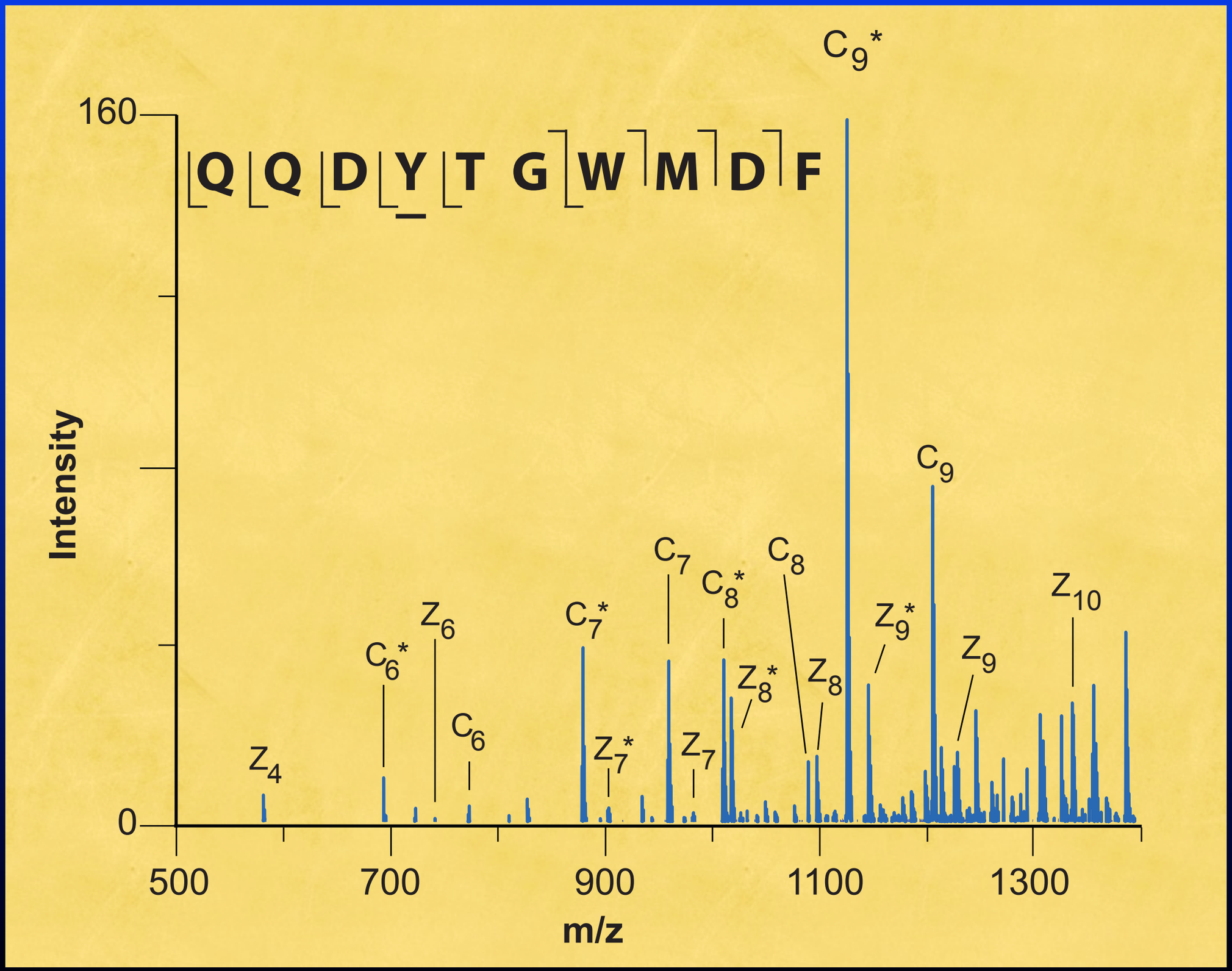
"No sulfated fragment was detected..." by ECD

"Sulfopeptides are more susceptible to neutral losses even under ECD conditions"

Medzihradszky et al., J am Soc Mass Spectrom, 2007

Evaluation of AP - ECD

Labile modifications #3: Sulfopeptide Caerulein

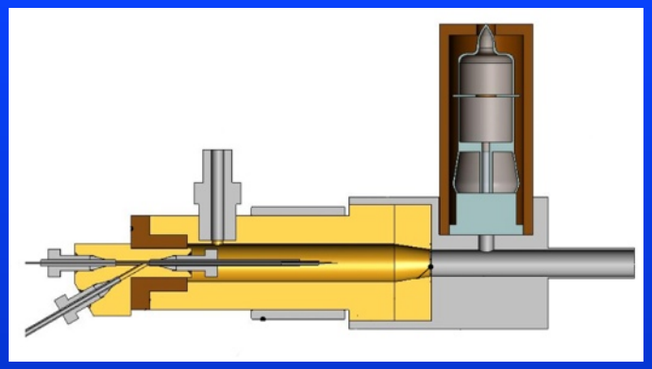


Atmospheric Pressure - Electron Capture Dissociation

AP-ECD Checklist

- ✓ Reproducible
- ✓ Sensitive
- ✓ Peptide mixtures
- ✓ Labile Modifications
  - ✓ glycopeptide
  - ✓ phosphopeptide
  - ✓ sulfopeptide

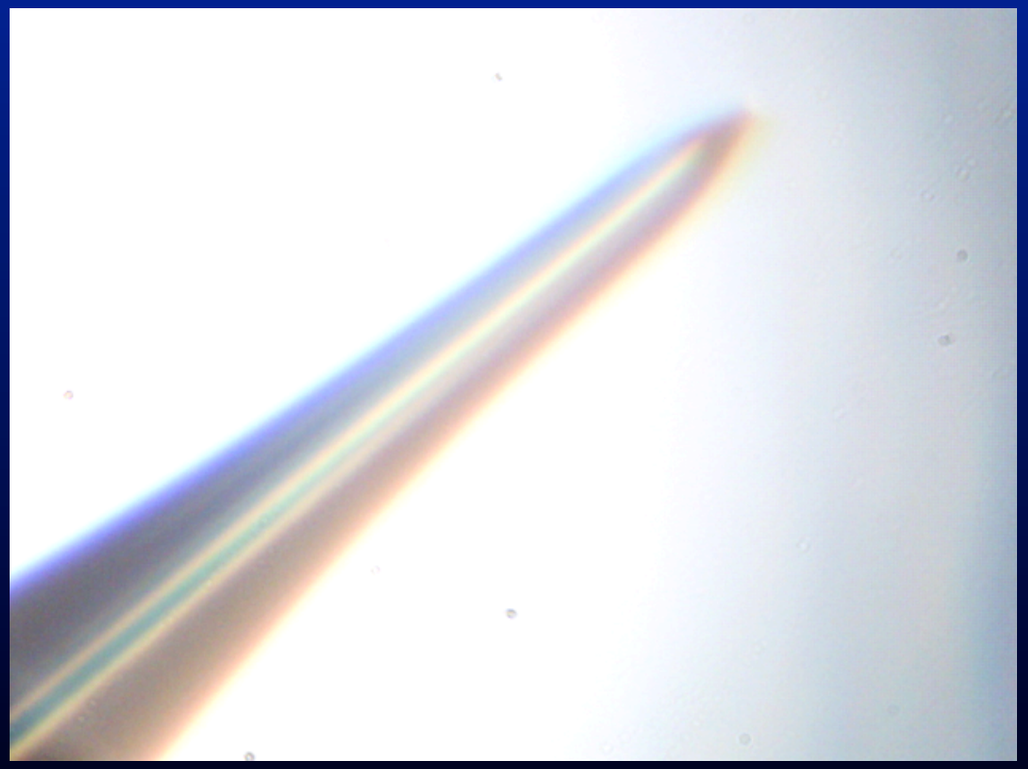
Hardware Modification #1
Different capillary tube



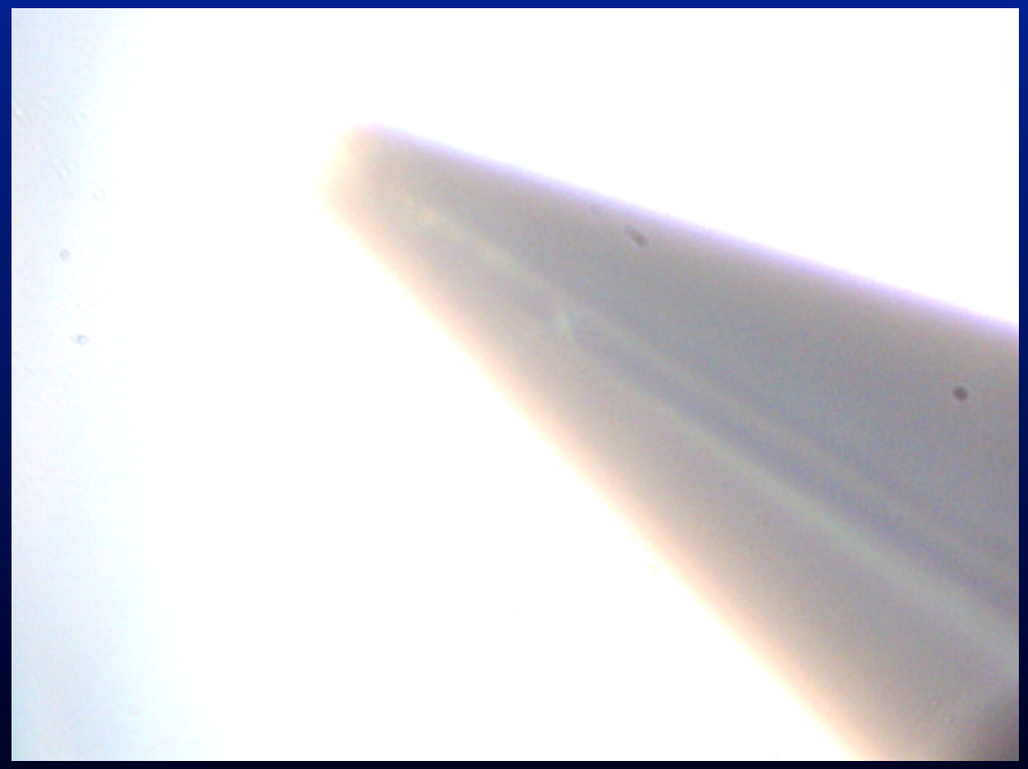
Problem: delicate tips would plug in days (solvent & debris)

Solution: Changed to 20 um constant ID (from 10 um taper tip)

Result: tips last for weeks (not days)
require faster flow > 1ul/min compared to 0.4 ul/min

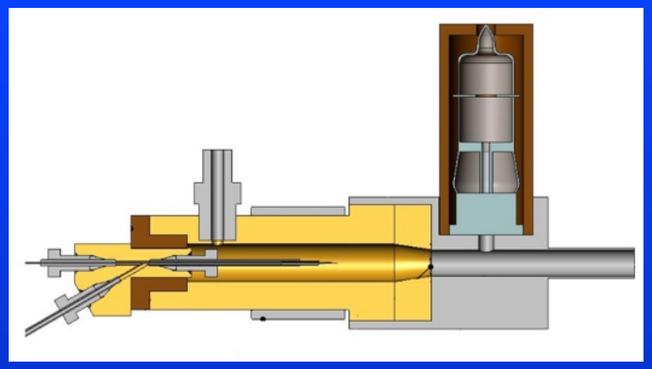


10 um taper tip



20 um const ID

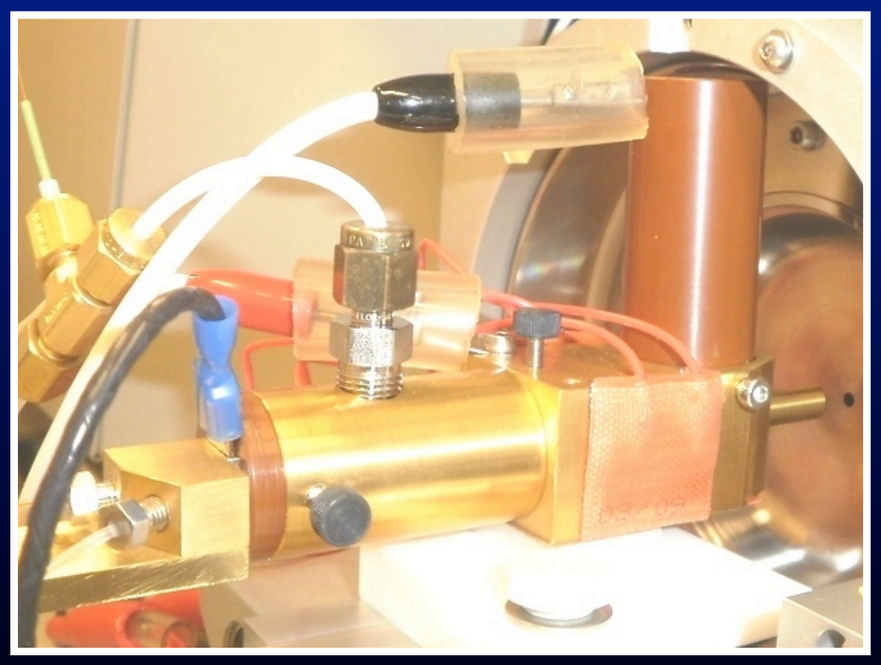
Hardware Modification #2
Programmable Heater



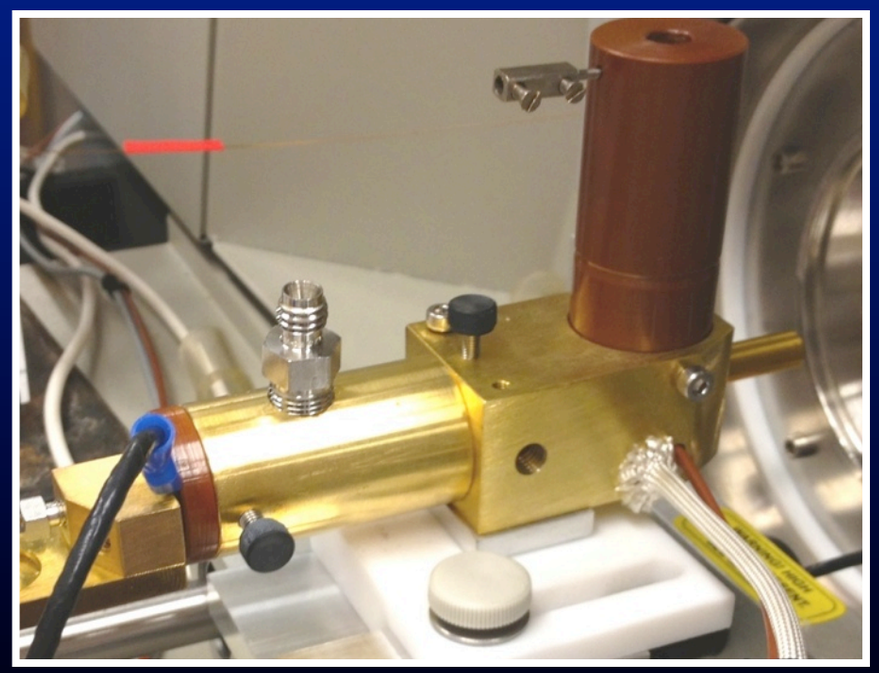
Problem: had to be turned off at night requiring subsequent daily
cleansing 1 hr bake outs
manual heater required attention to avoid burnout

Solution: Changed to programmable heater (previously manual heater)

Result: daily morning 1 hr cleansing bakeouts eliminated
less operator intervention to maintain constant temp

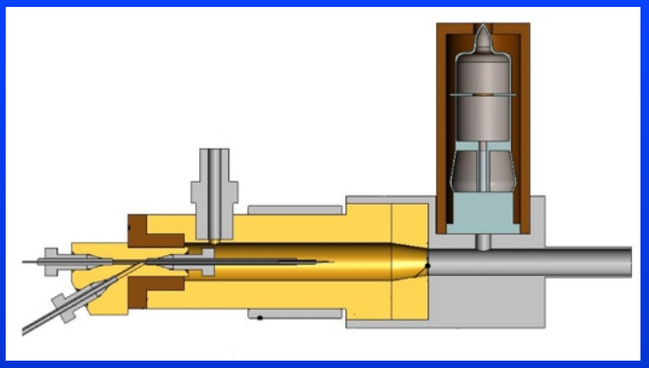


Old pad heater



New block heater

Hardware Modification #3
Spacers



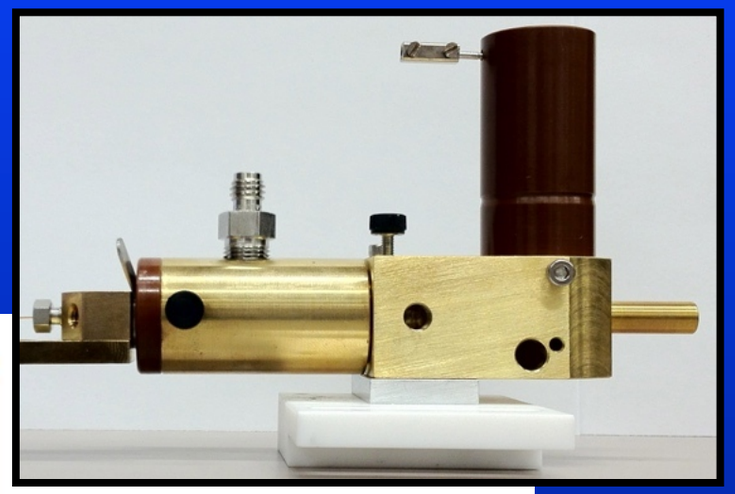
Problem: slow degradation of early eluting low charge ECD fragments

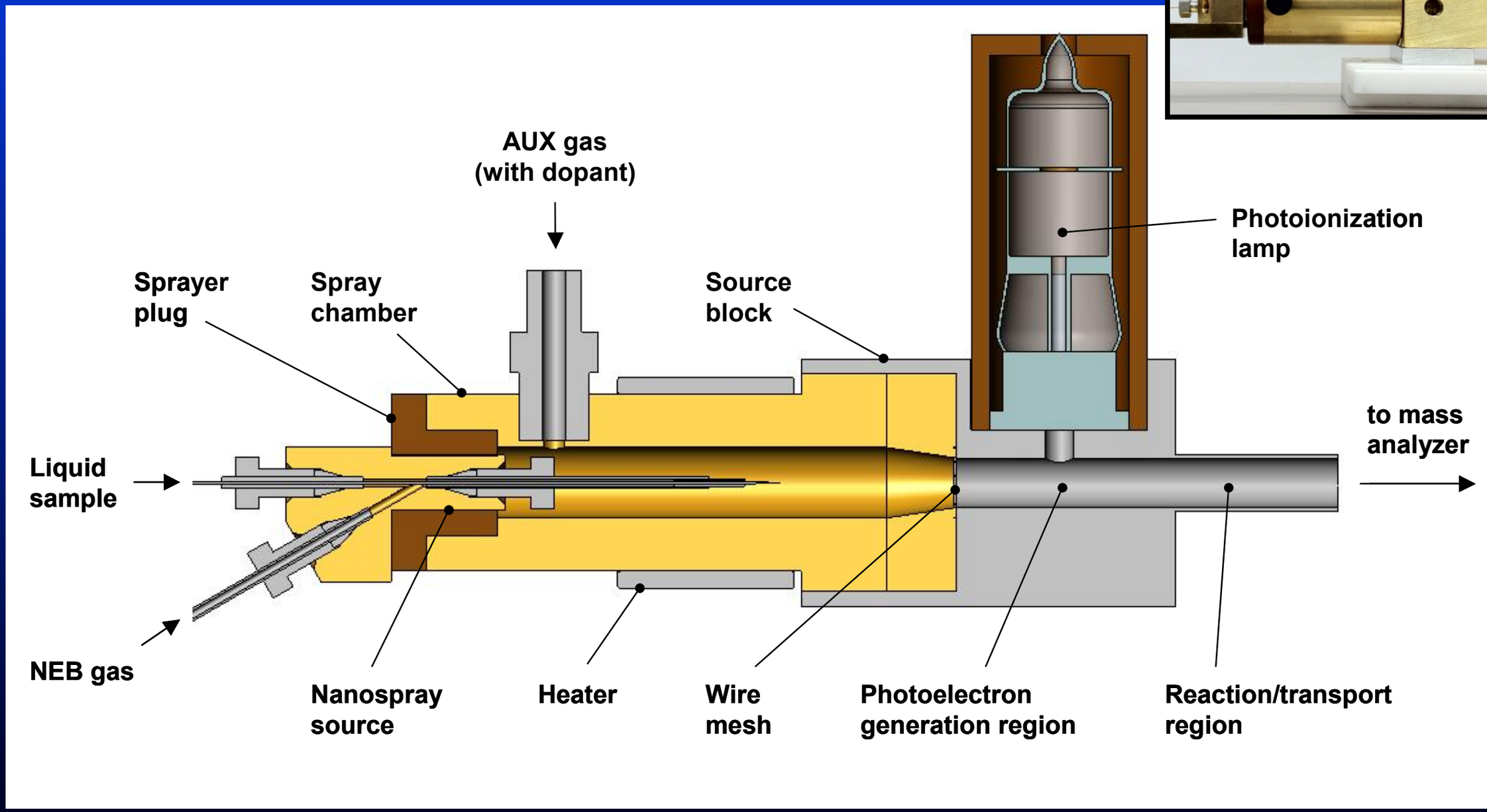
Cause: wire electrode mesh becoming dirty overtime

Solution: increase distance from emitter & mesh

Atmospheric Pressure - Electron Capture Dissociation

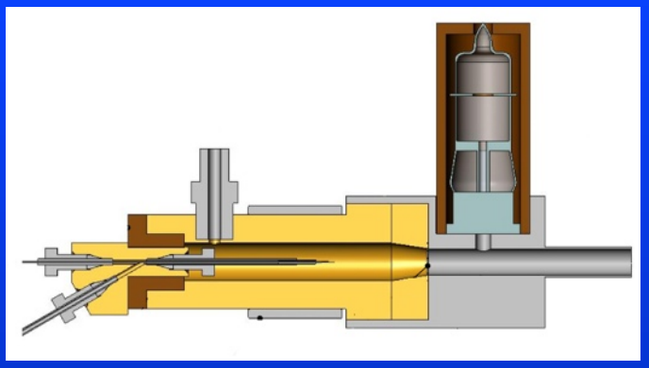
How it works?





$$\text{acetone}\ \text{IE 9.7 eV} + h\nu\ 10\ \text{eV} \rightarrow \text{acetone}^{+} + e^{-}\ \text{KE=0.3 eV}$$

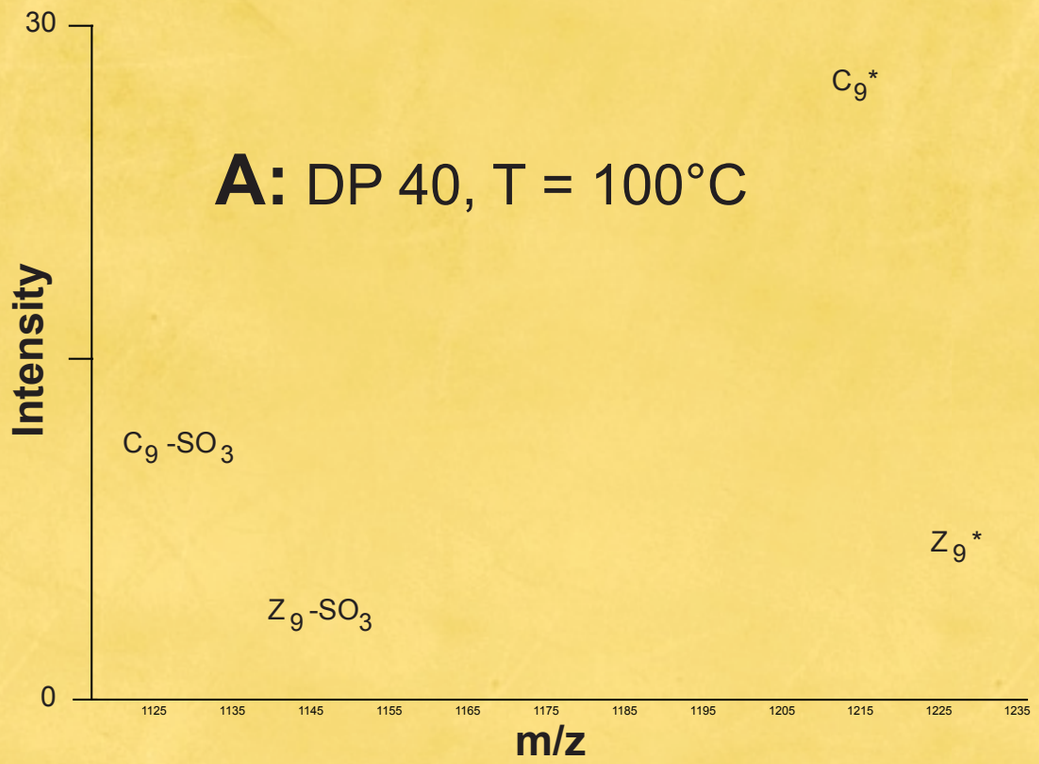
Hardware Modification #3
Spacers



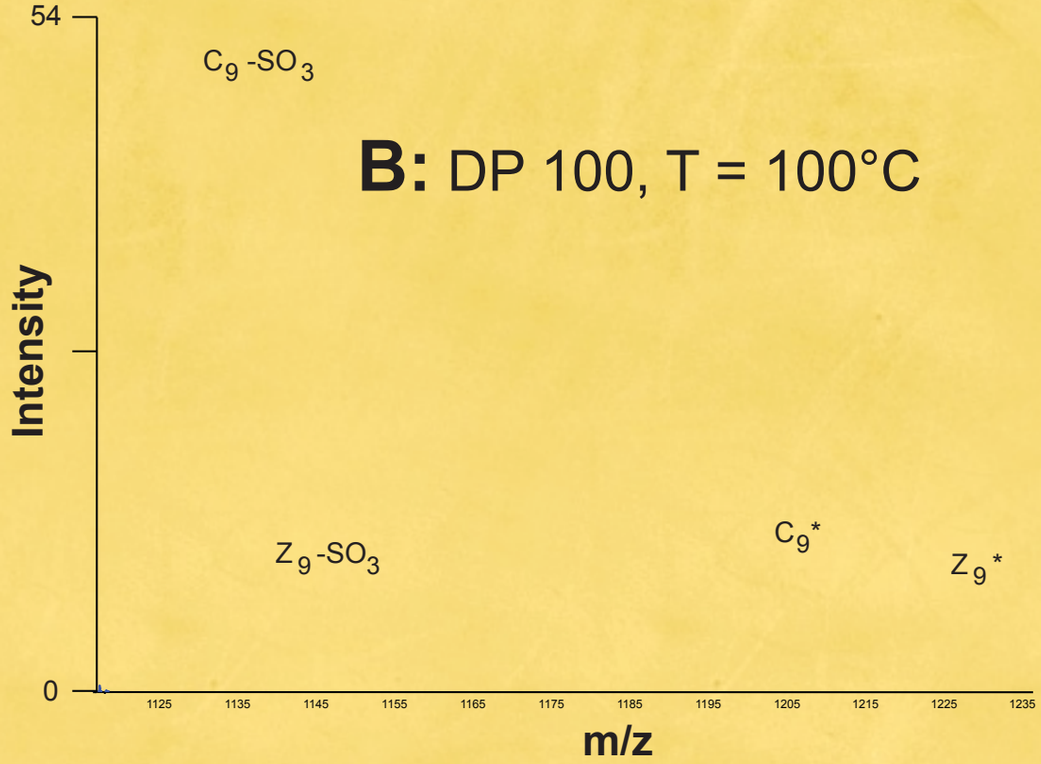
Experiment: Tested no grill electrode, 1 cm, 2 cm & 3 cm spacers (with and without mesh electrode)

Result: found that original mesh setup could go for > 1 week without loss of signal

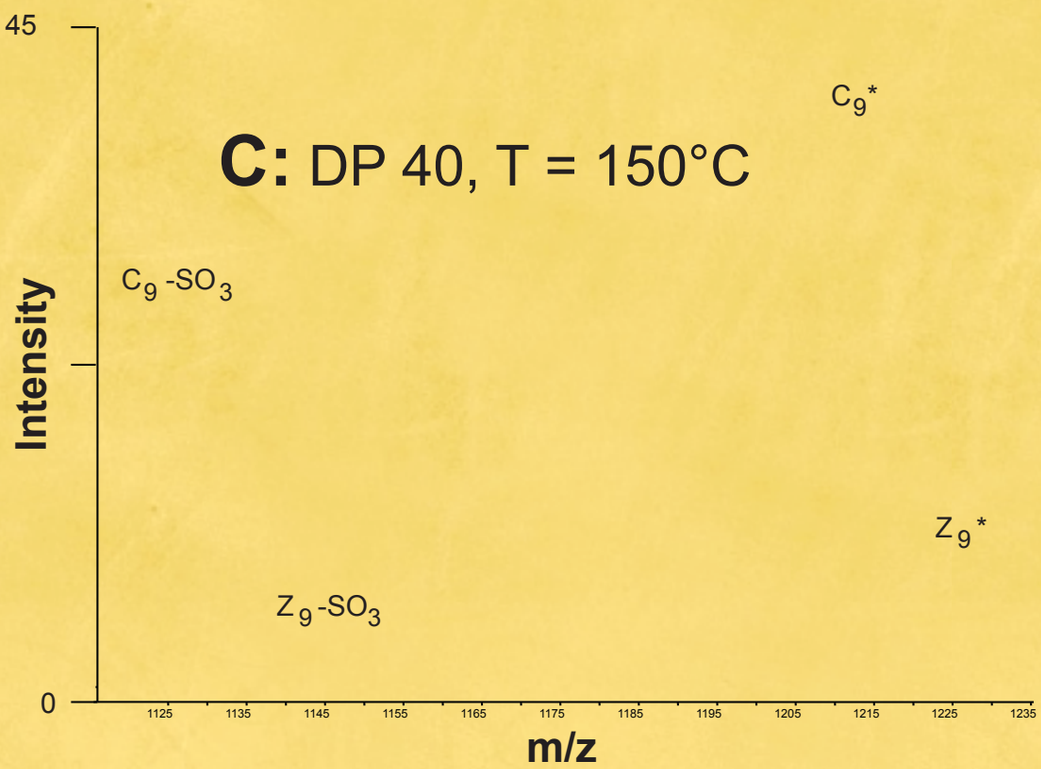
AP-ECD Parameters



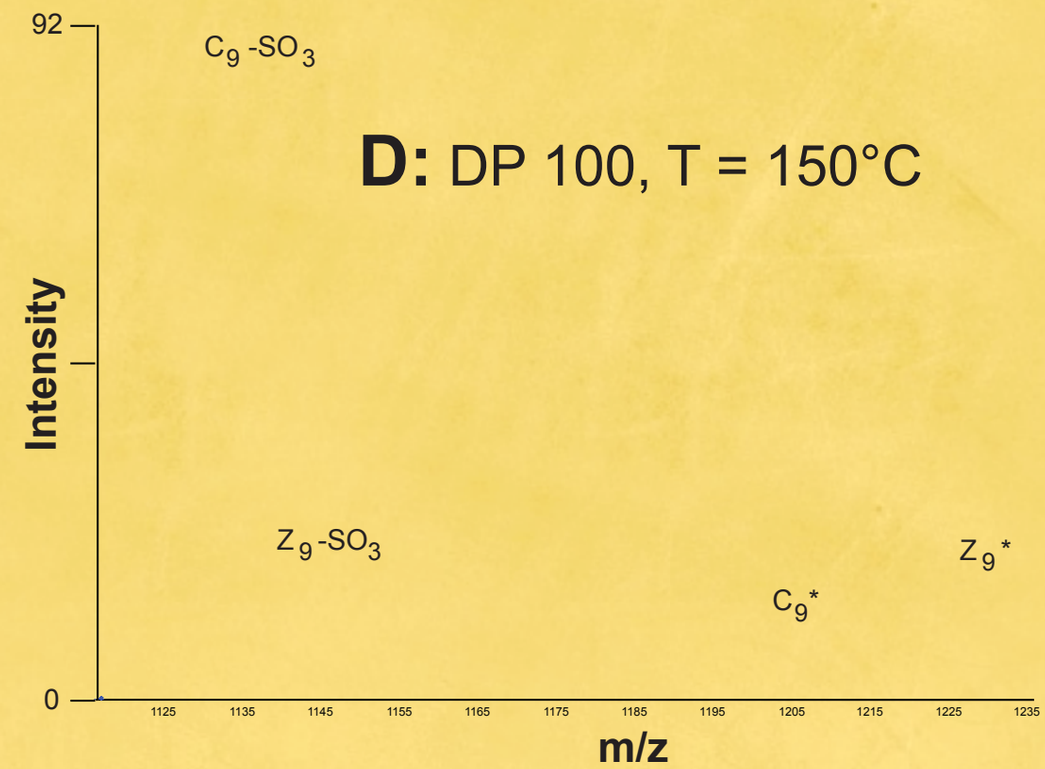
A: DP 40, T = 100°C



B: DP 100, T = 100°C

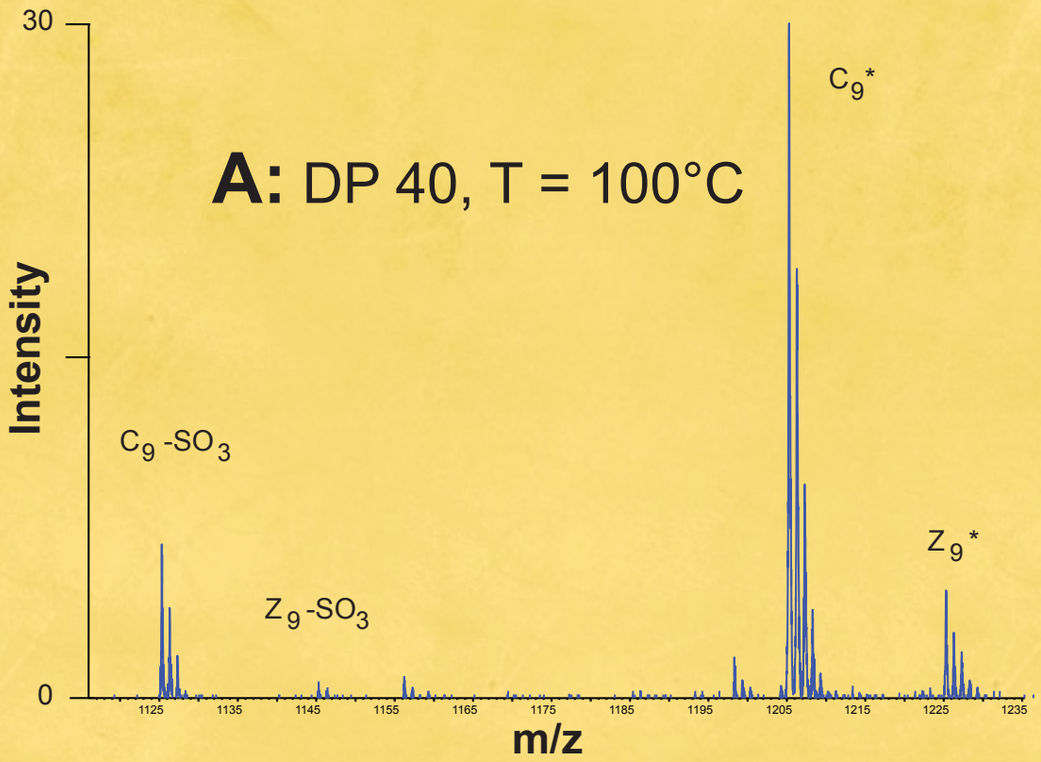


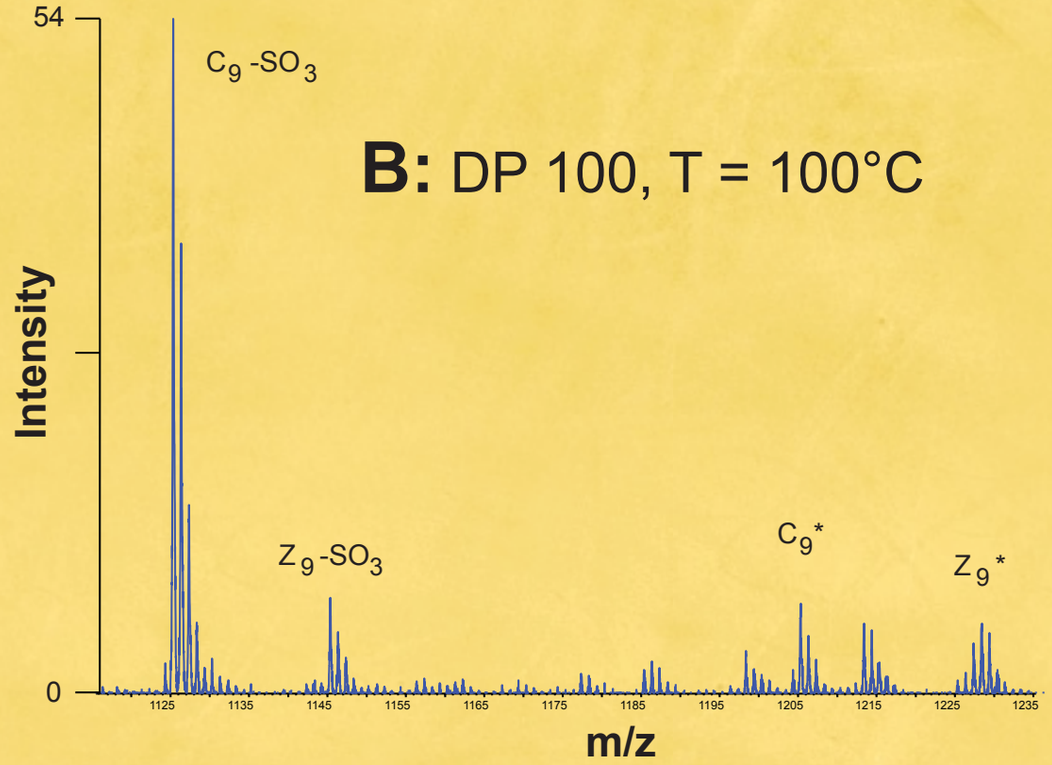
C: DP 40, T = 150°C

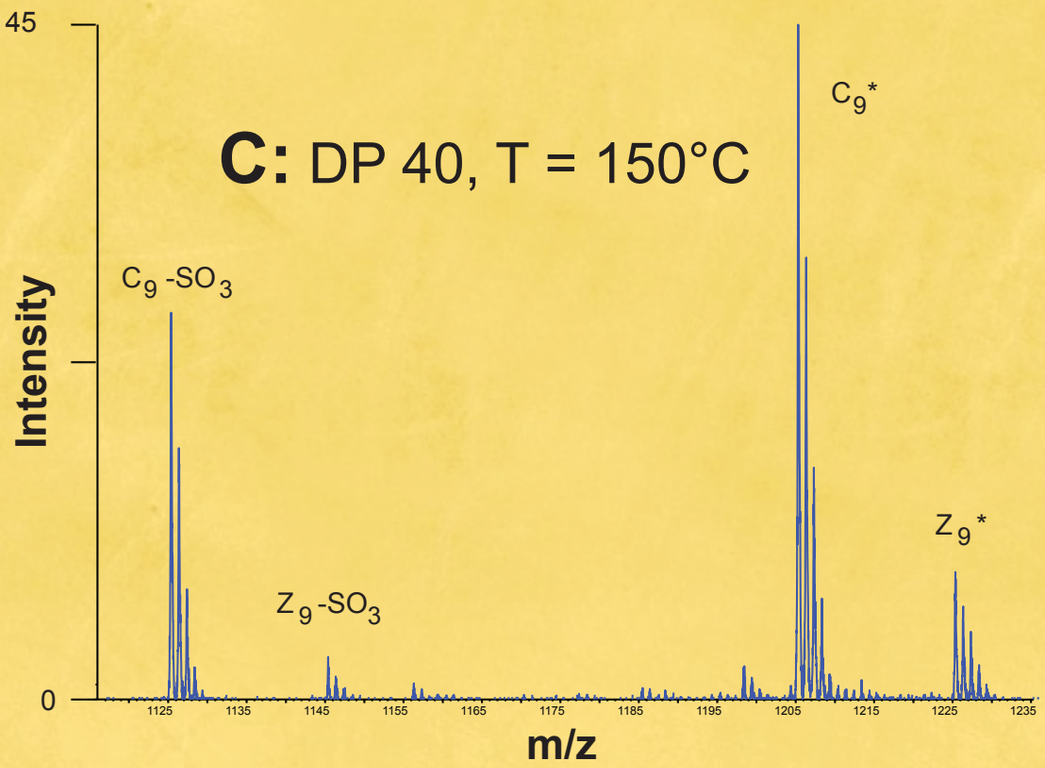


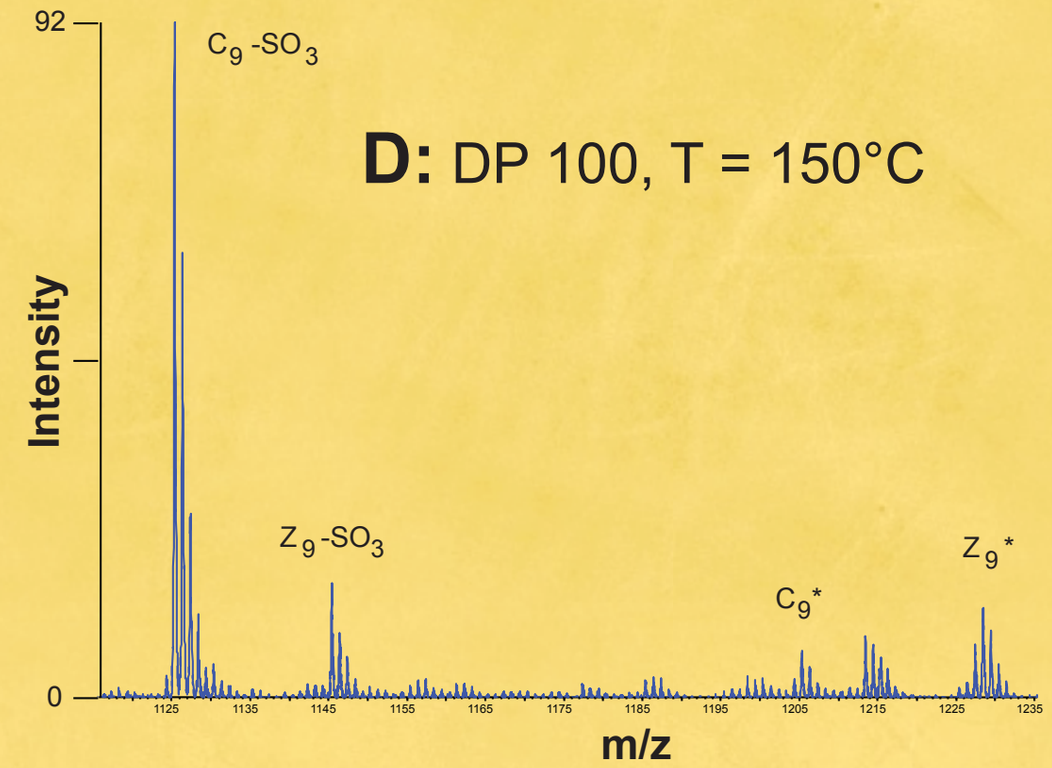
D: DP 100, T = 150°C

AP-ECD Parameters

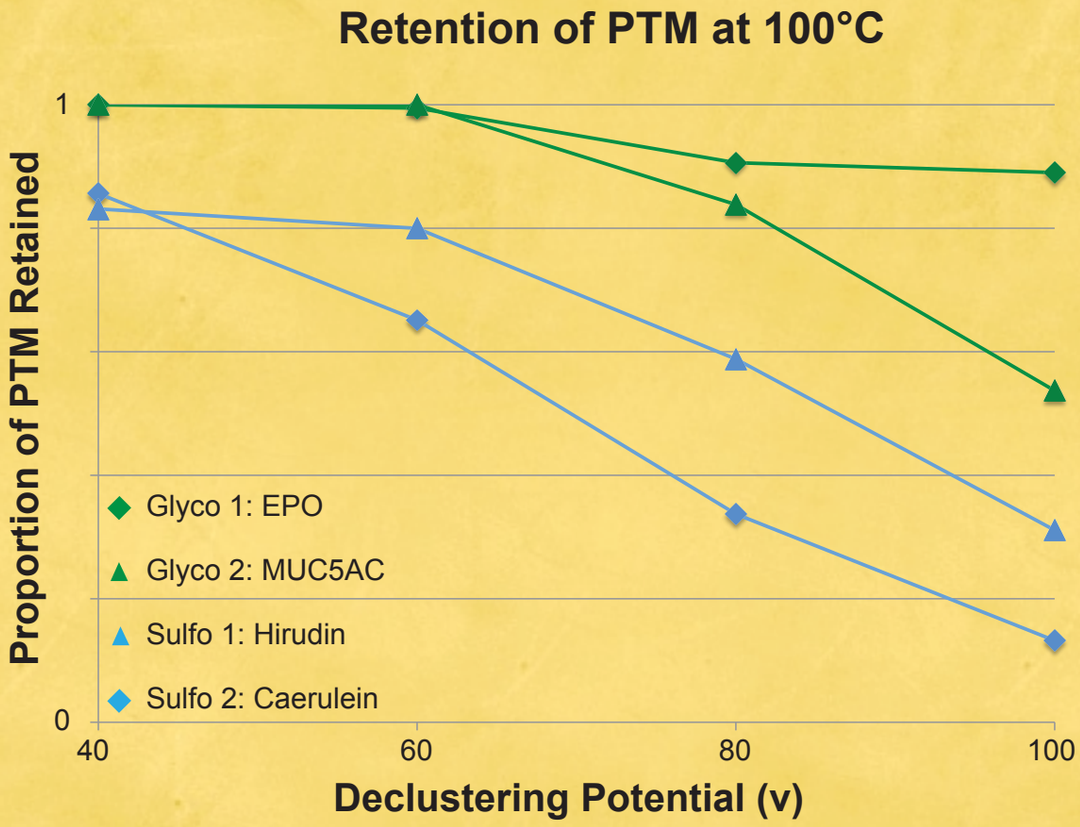


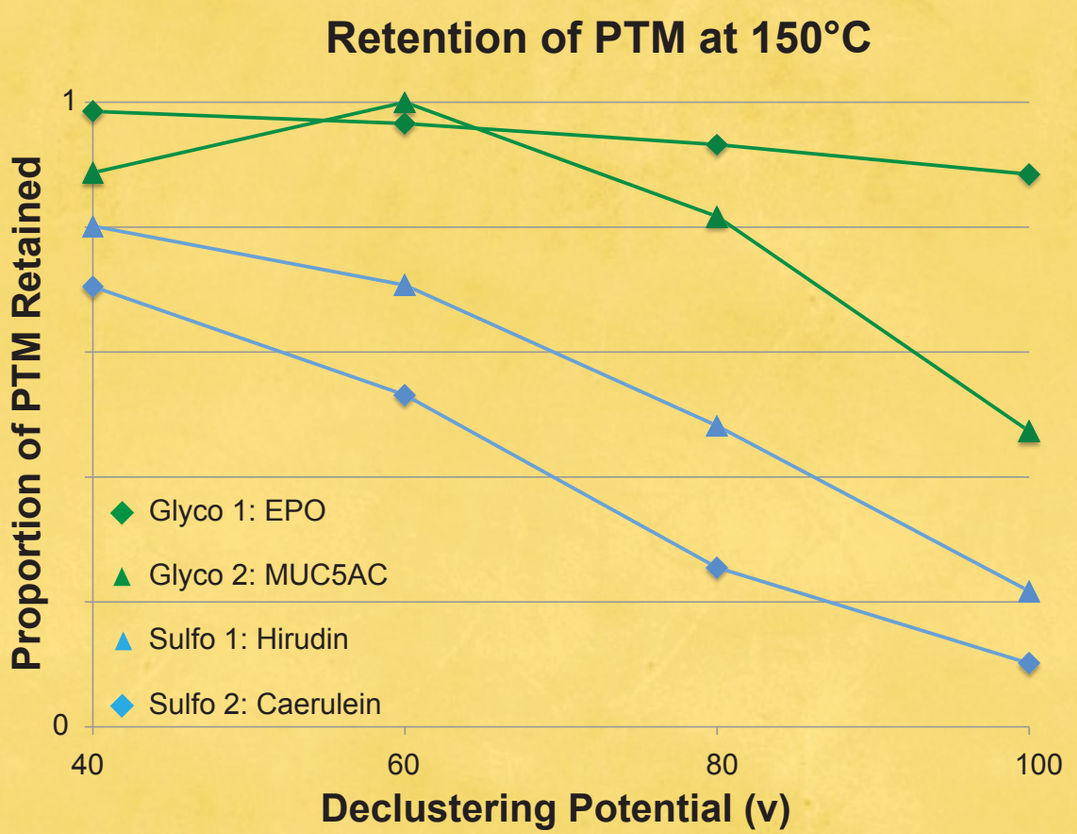






AP-ECD Parameters

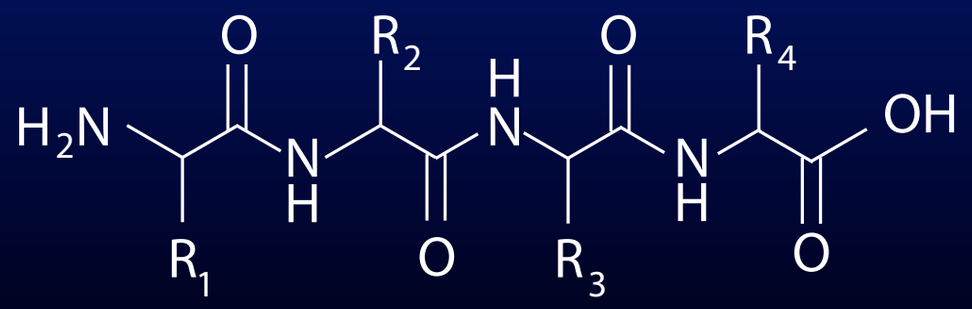




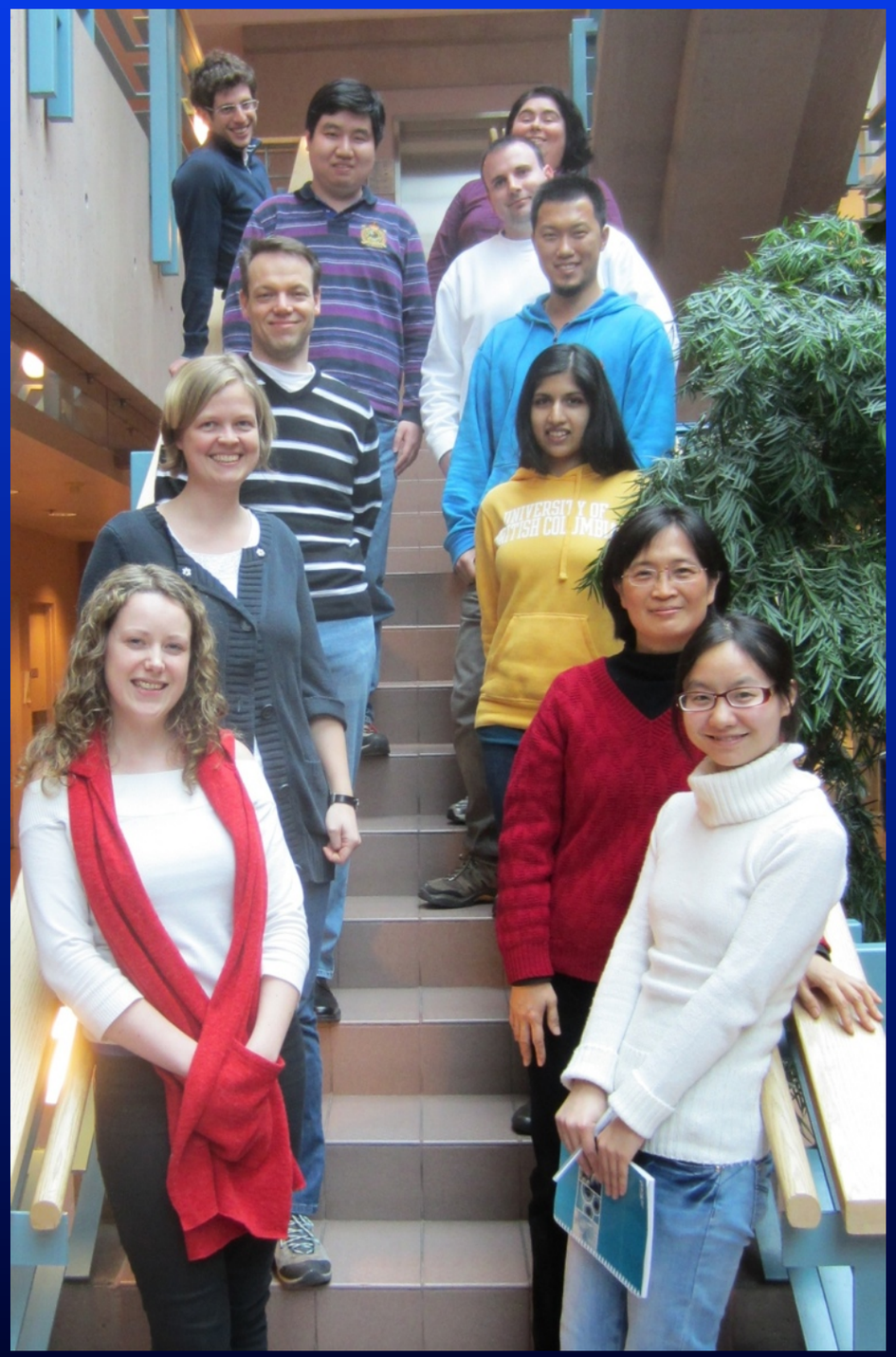
AP-ECD
take away

- retro fitted to any API instrument
- useful for structural analysis of peptides with labile modifications, simple mixtures

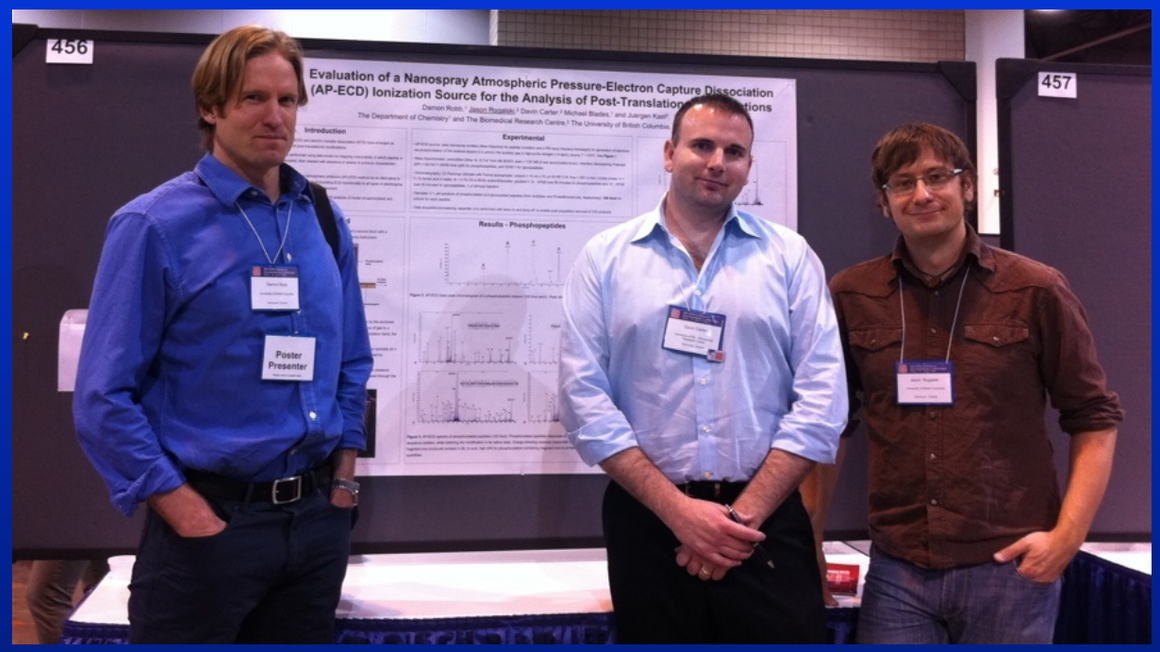
AP-ECD outside MS therefore no mass separation



Acknowledgements



Juergen Kast Lab
UBC



Damon Robb

Jason Rogalski

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

Thanks for listening.....

Thank you:

Meng-Qiu
UppCon Organizing Committee
Sponsors

a place of mind

UppCon '13
Feb. 18, 2013

Questions?

a place of mind

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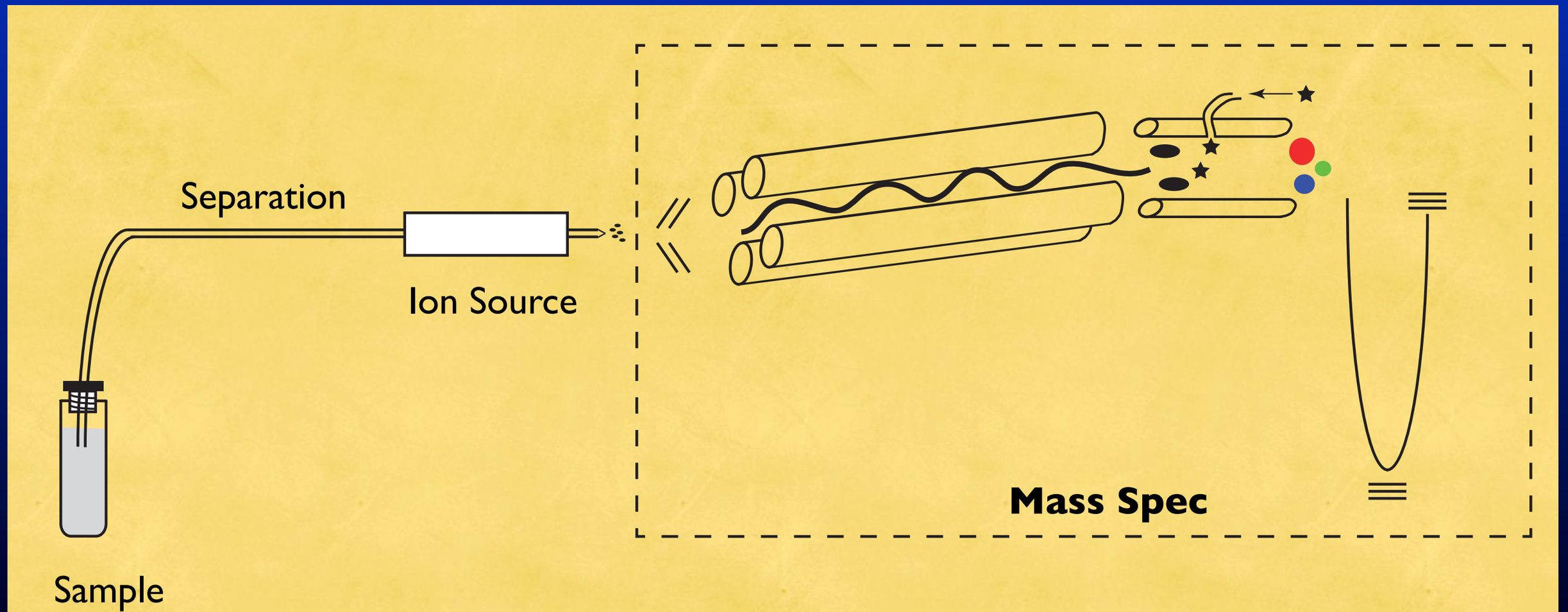
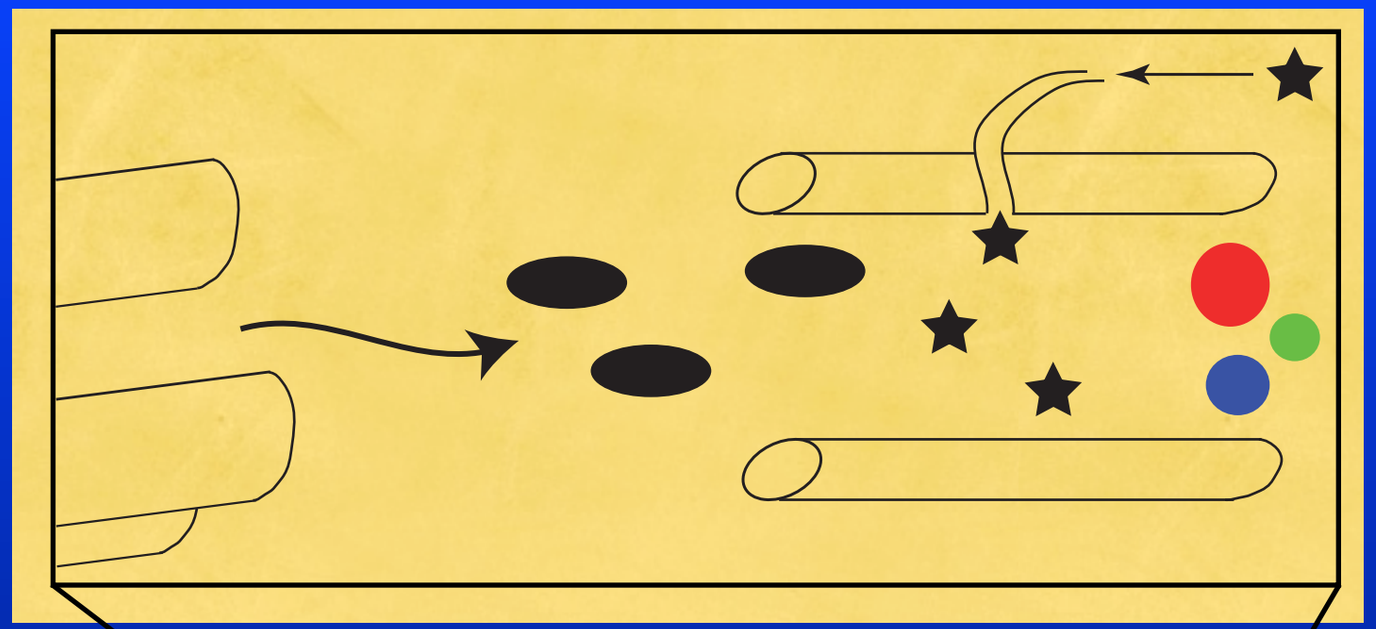
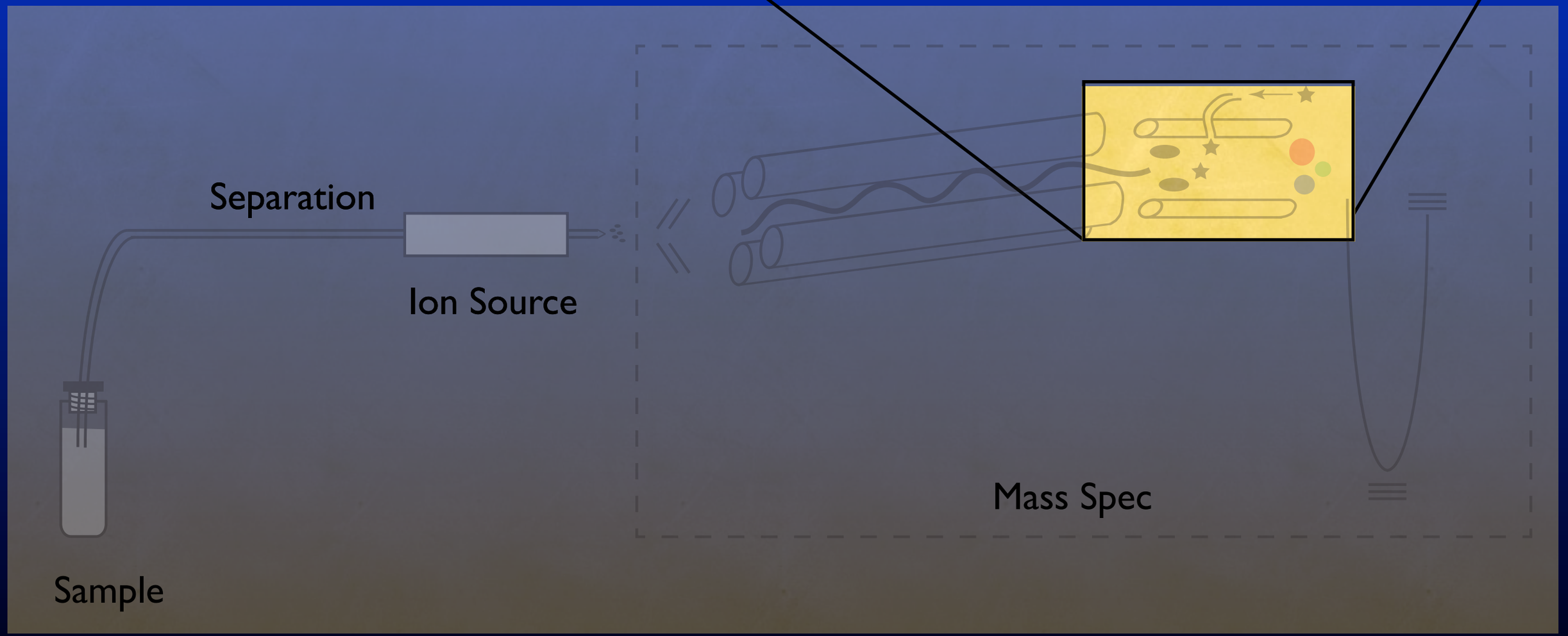
Mass Spectrometry
all about moving ions



Mass Spectrometry
How it is usually done

Collision Induced Dissociation

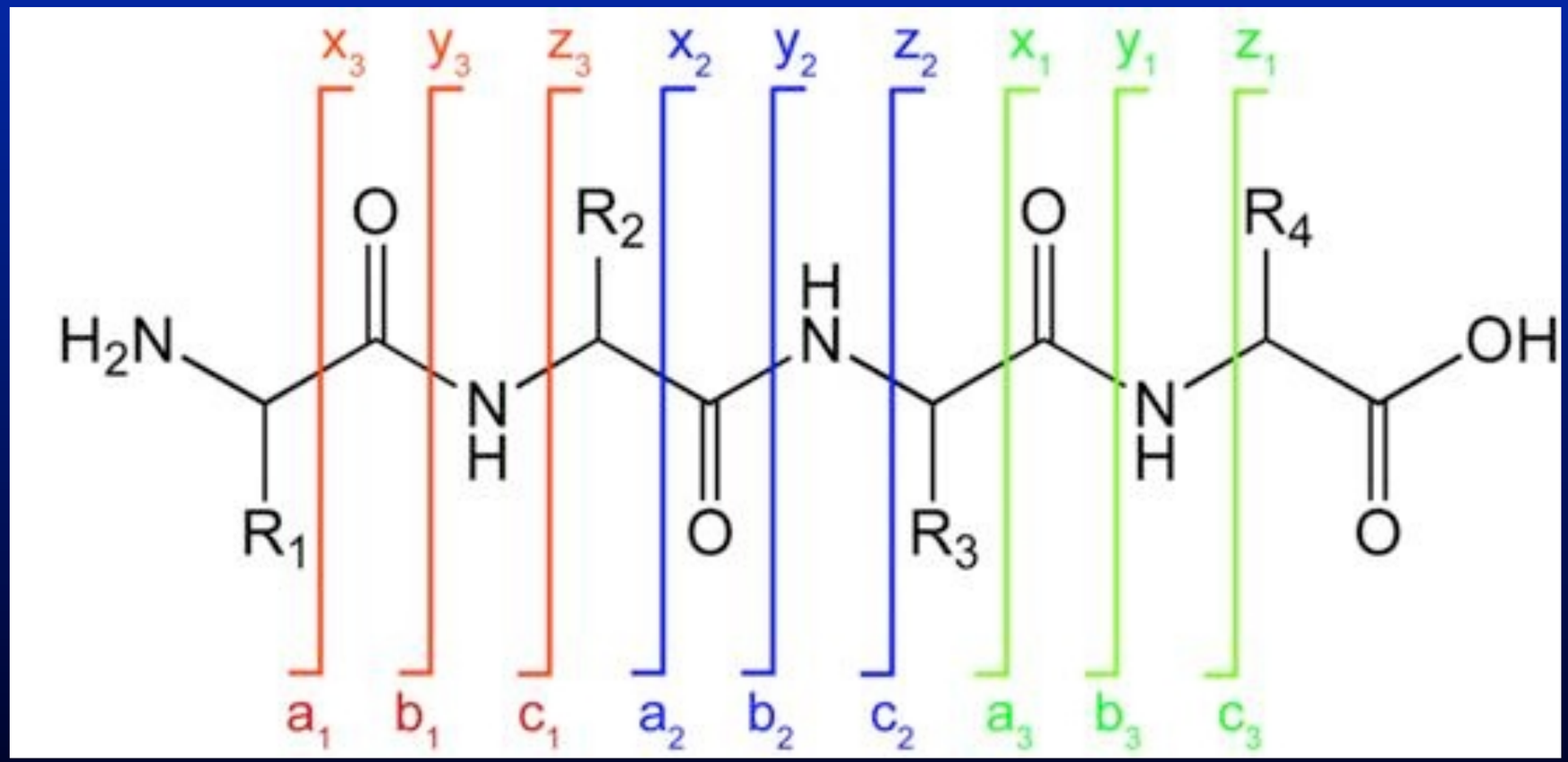




Atmospheric Pressure - Electron Capture Dissociation

Why?

Add ECD capacity on our instrument
- fuller coverage
- localize labile modifications



b & y ions: Collision Induced Dissociation
c & z ions: Electron Capture Dissociation

Proteomics

Understanding cause of diseased states

Study of all proteins in a system

Focused on blood research around cardiovascular disease and cancer

Post translational modifications

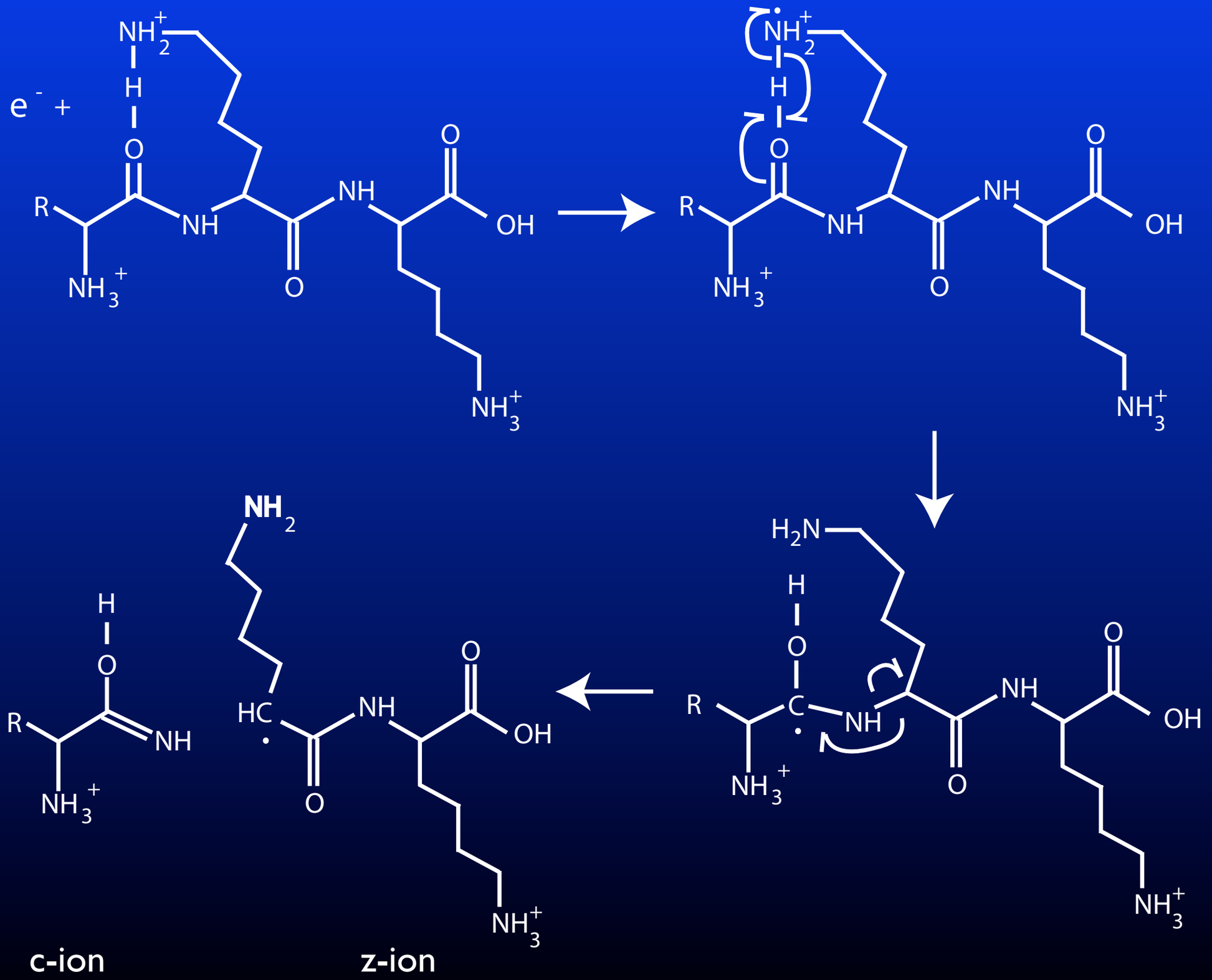
> 300 chemical modifications after translation

Identify and localize

Modification
5' dephospho
Desmosine (from Lysine)
decomposed carboxymethylated Methionine
Ornithine (from Arginine)
Lysinoalanine (from Cysteine)
Lanthionine (from Cysteine)
Dehydroalanine (from Cysteine)
Homoserine formed from Met by CNBr treatment
Dehydration (-H2O)
S-gamma-Glutamyl (crosslinked to Cysteine)
O-gamma-Glutamyl- (Crosslink to Serine)
Serine to Dehydroalanine
Alaninohistidine (Serine crosslinked to theta or pi
Pyroglutamic Acid formed from Gln

Mass Spectrometry - Electron Capture Dissociation

ECD Mechanism



Mass Spectrometry - Electron Capture Dissociation

ECD Mechanism