Kast Lab Seminar

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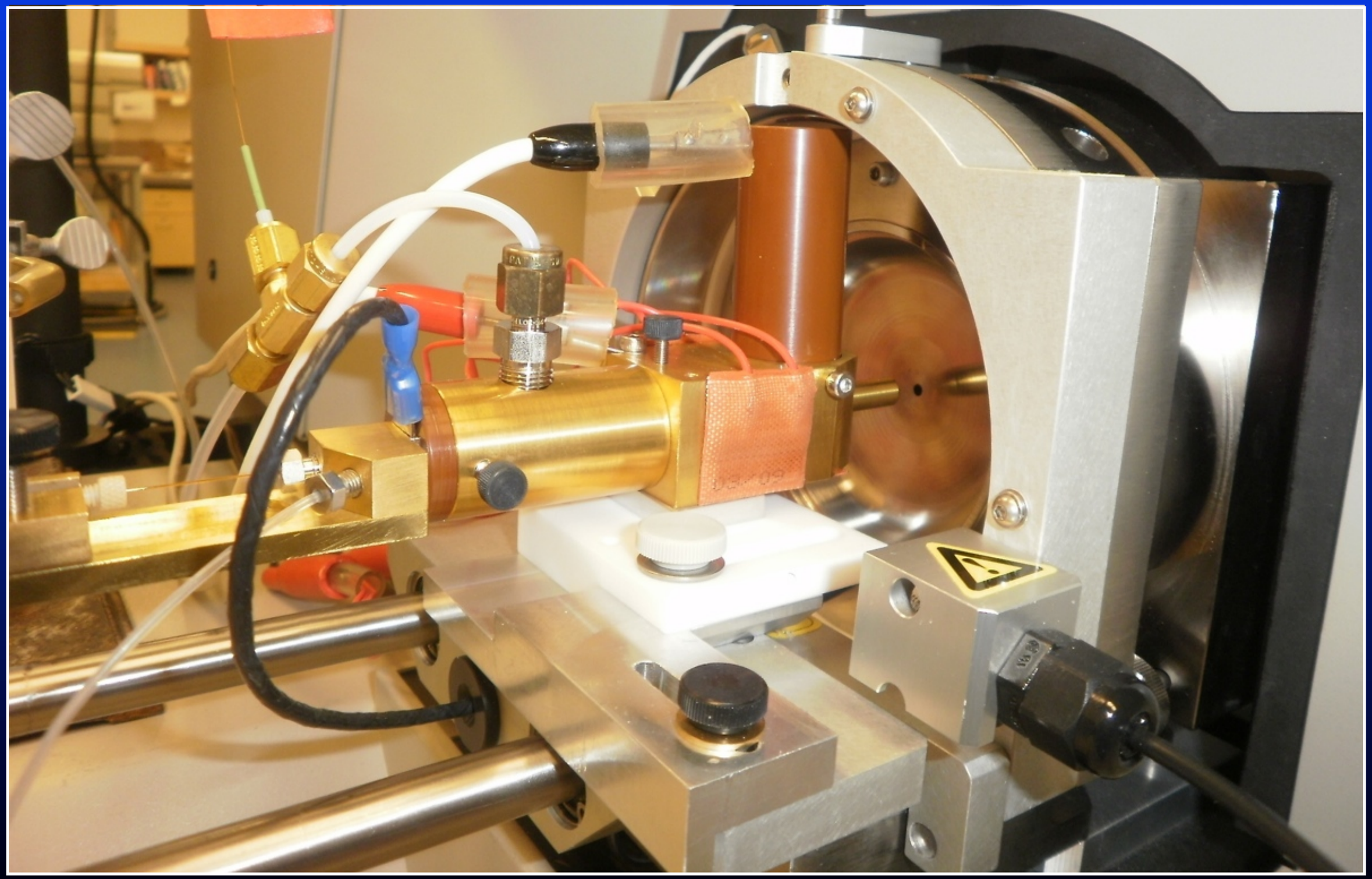
Biomedical Research Centre

Dec. 6, 2011

a place of mind

Atmospheric Pressure - Electron Capture Dissociation
Mass Spec hardware design - like a plumber on a small scale



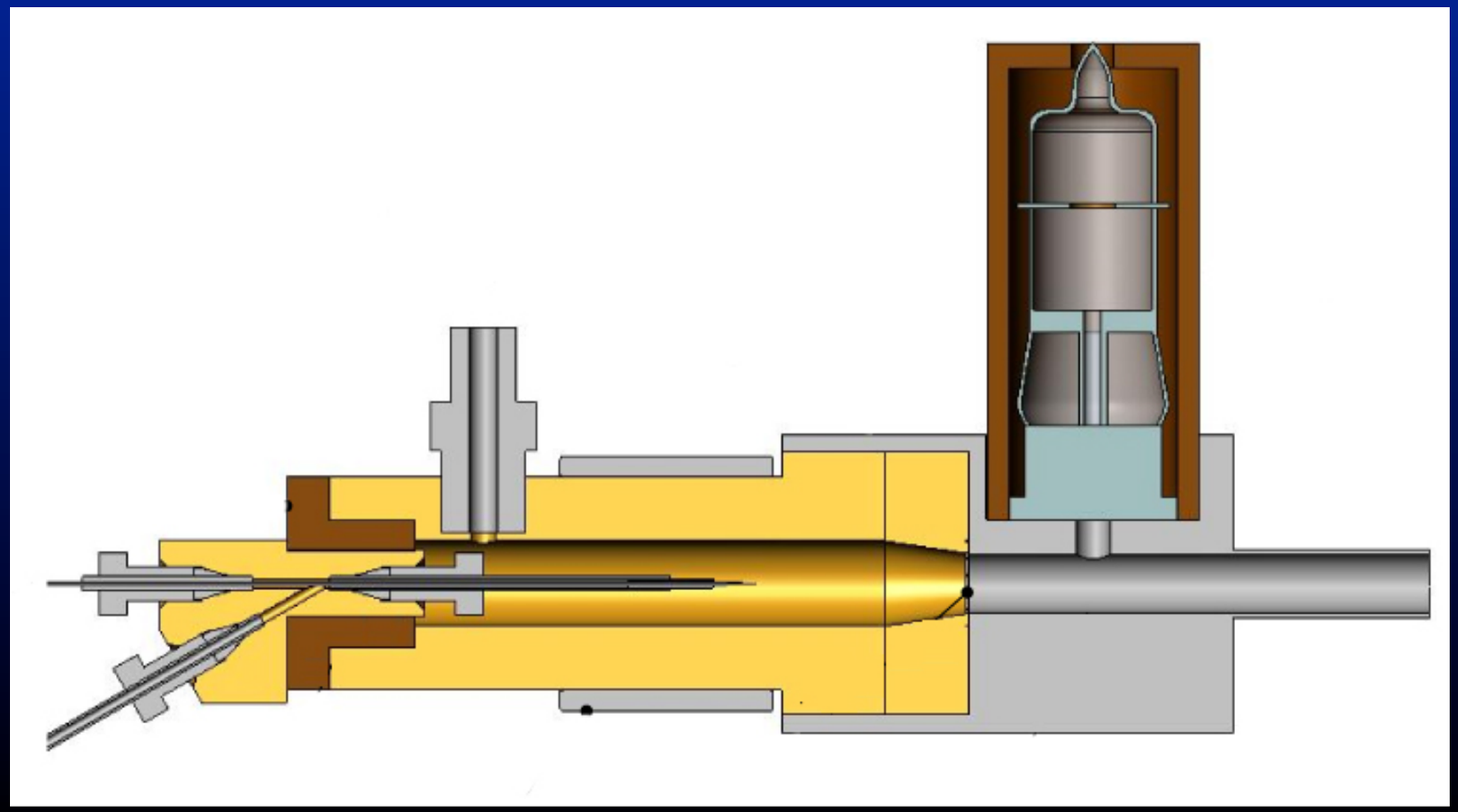
Atmospheric Pressure - Electron Capture Dissociation
Where we where in the Summer

< 1 fmol LOD on Substance P

BSA mixture using chromatography

Reproducible, 1 day

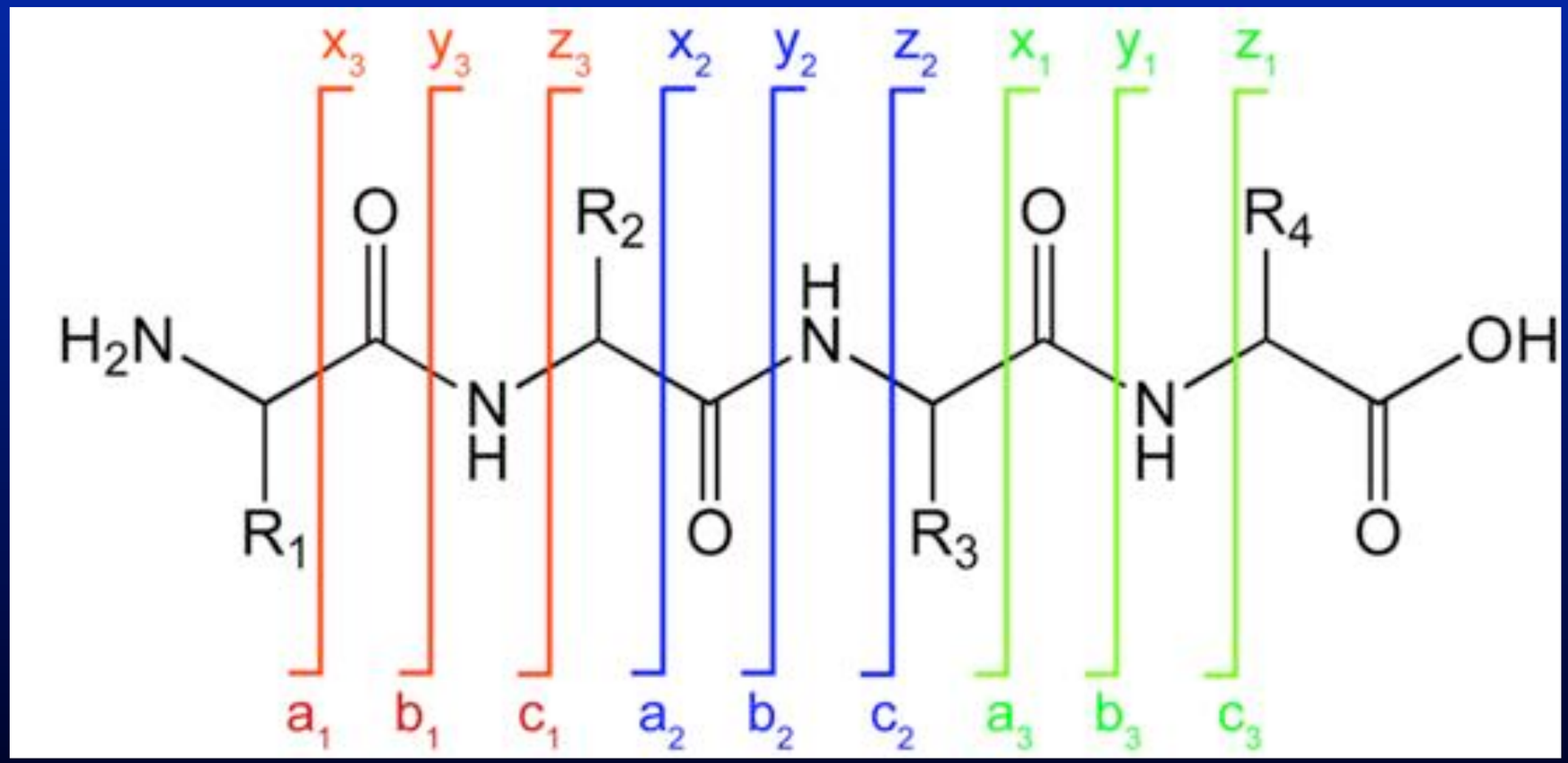
Localize glycosylation & phosophopeptide



Atmospheric Pressure - Electron Capture Dissociation

Why?

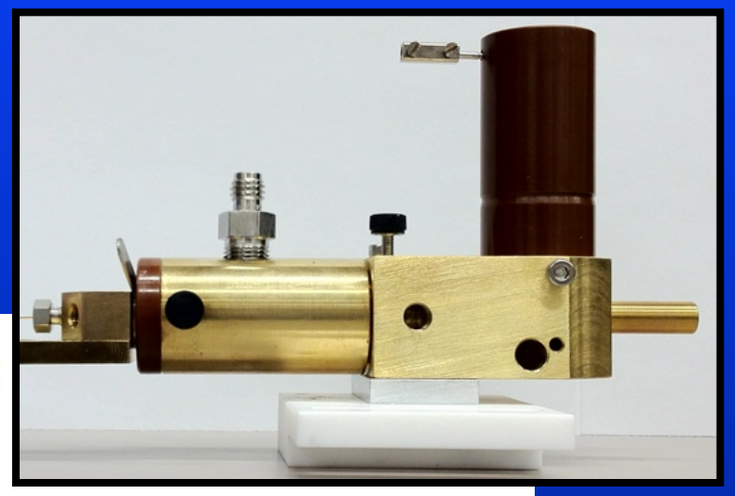
Add ECD capacity on our instrument
- fuller coverage
- localize labile modifications

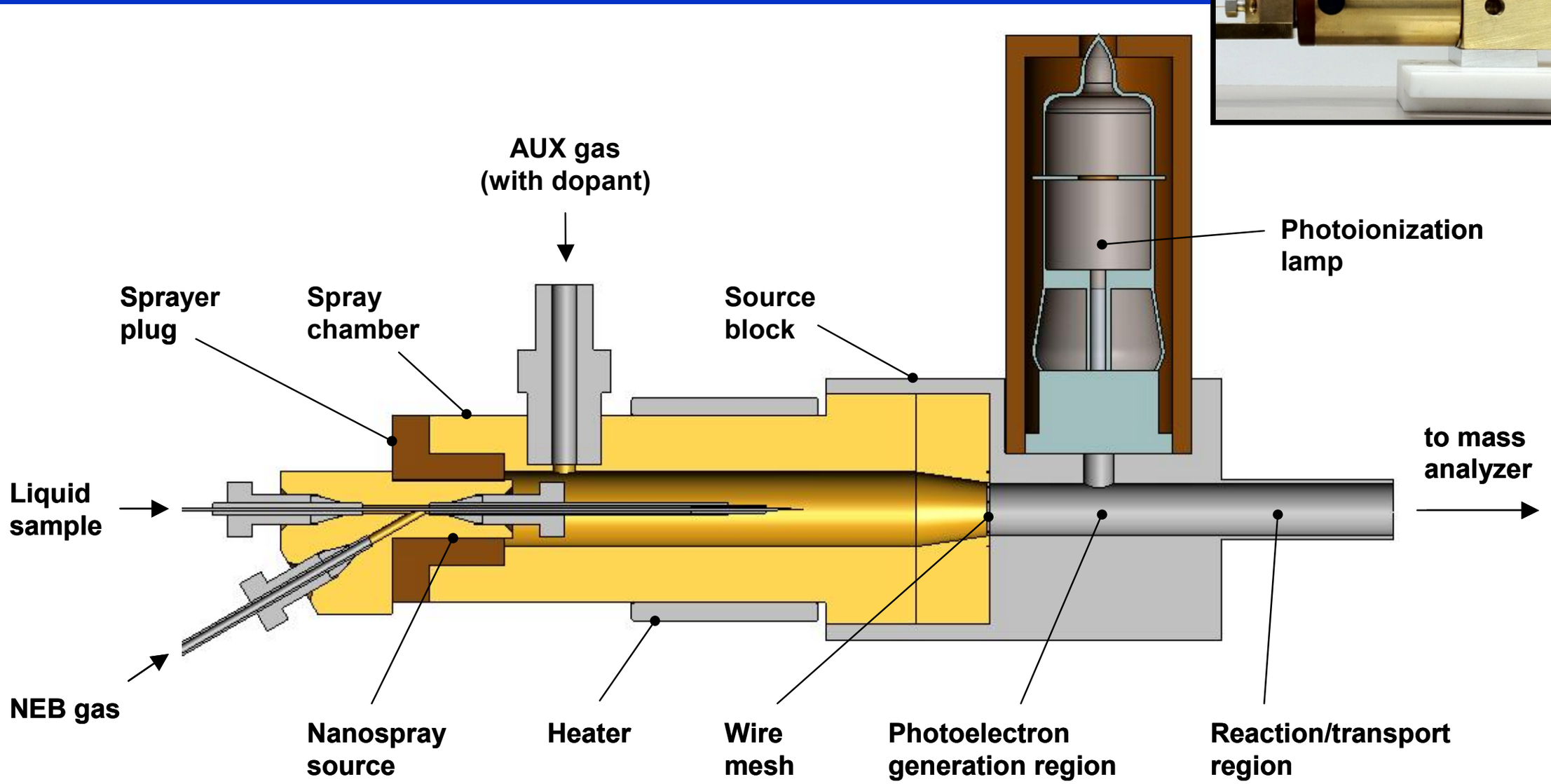


B & Y ions: Collision Induced Dissociation
C & Z ions: Electron Capture Dissociation

Atmospheric Pressure - Electron Capture Dissociation

How?

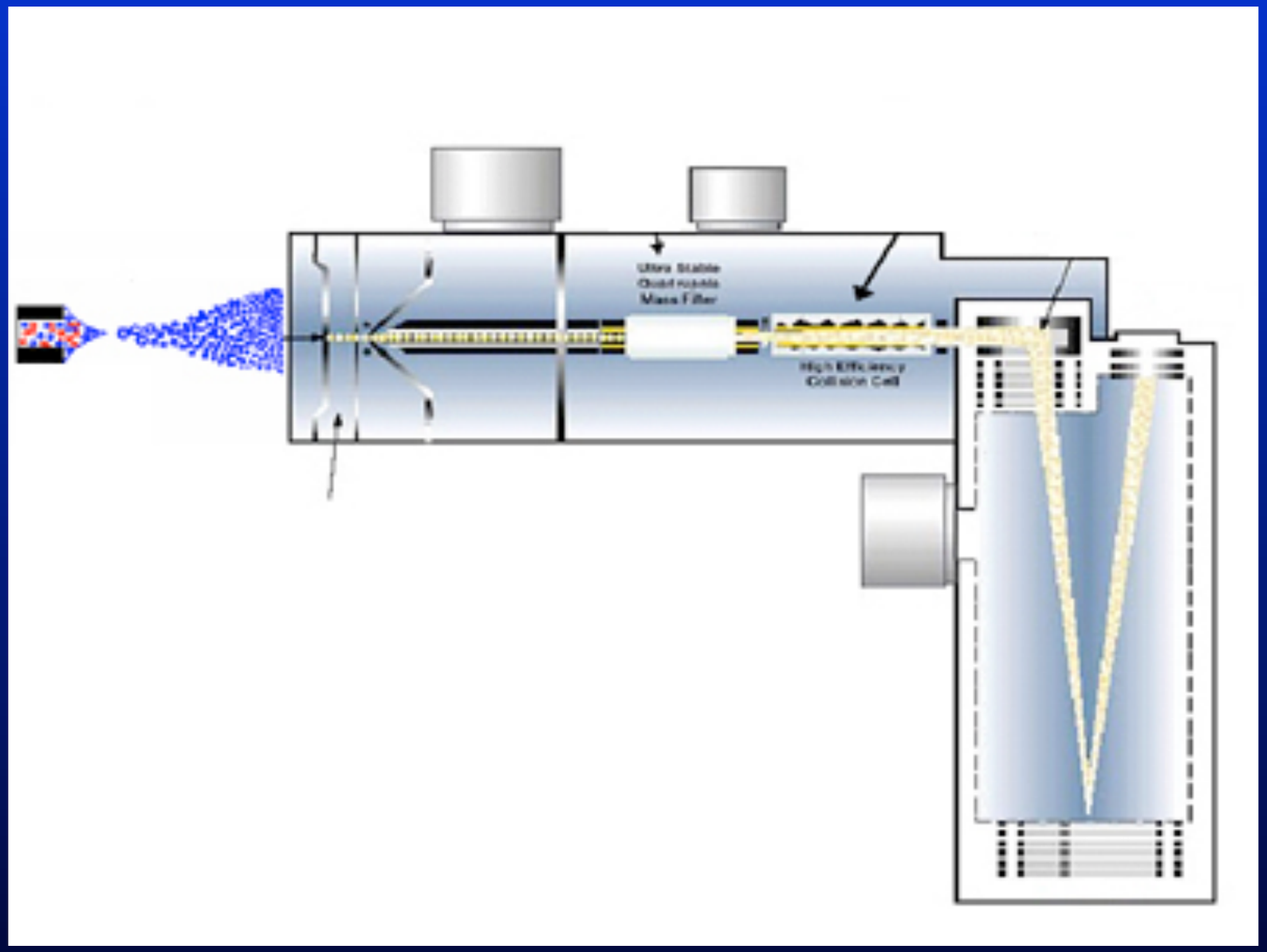




$$acetone_{IE\ 9.7\ eV} + h\nu_{10\ eV} \rightarrow acetone^{+} + e^{-}_{KE=0.3\ eV}$$

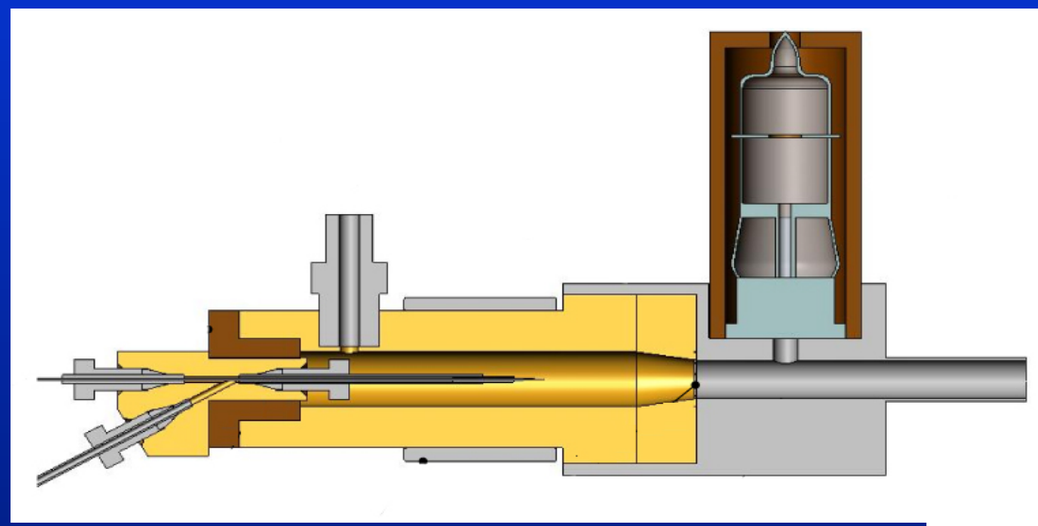
Atmospheric Pressure - Electron Capture Dissociation

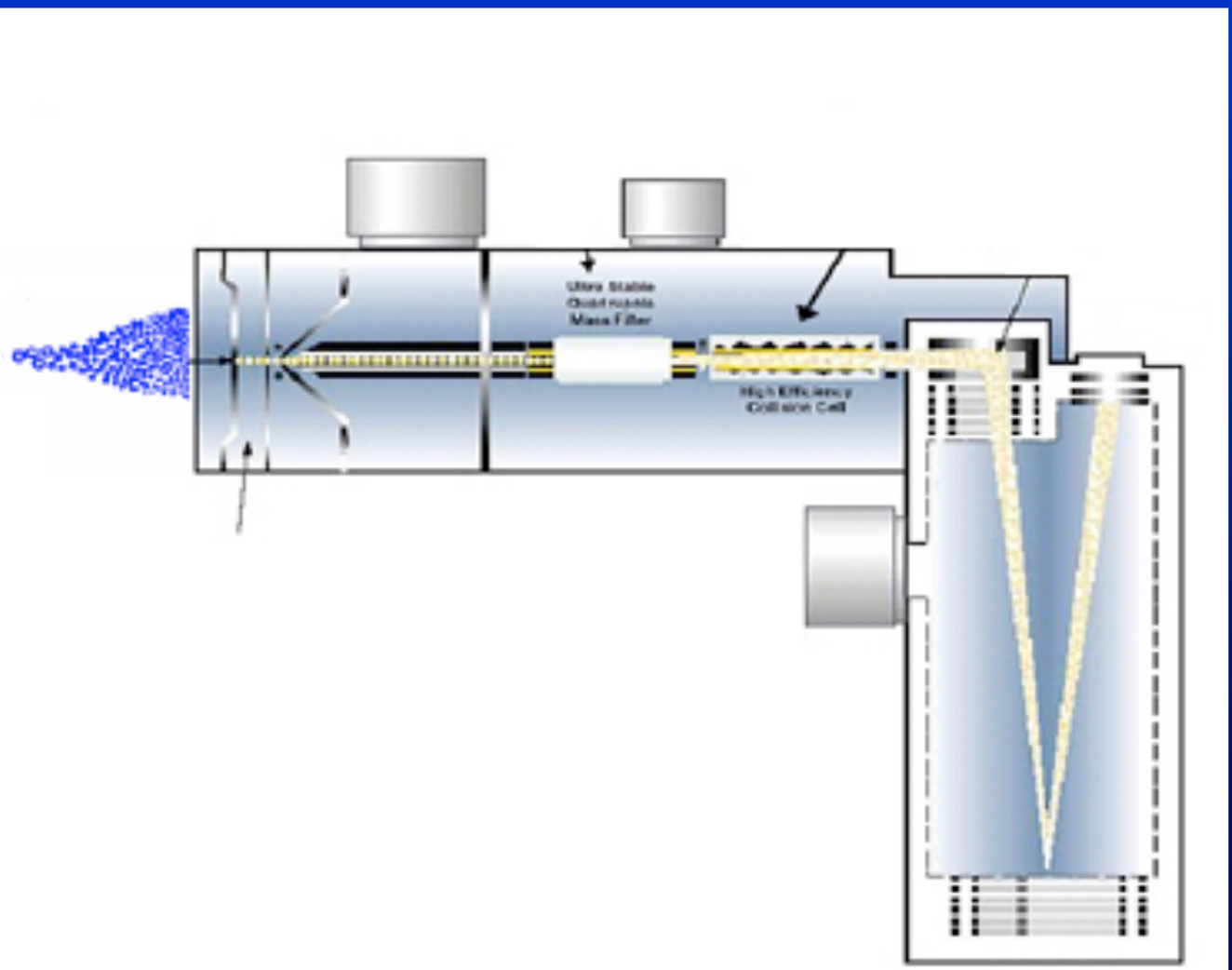
Where is goes



Q CID TOF

Atmospheric Pressure - Electron Capture Dissociation
Where is goes





nESI AP-ECD Q CID TOF

Atmospheric Pressure - Electron Capture Dissociation

Goals

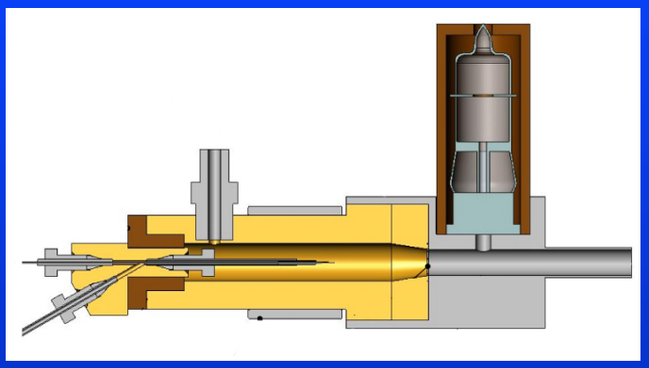
Commercialize

make it robust (sensitive, reliable, turn-key)

Generate biological data

Atmospheric Pressure - Electron Capture Dissociation

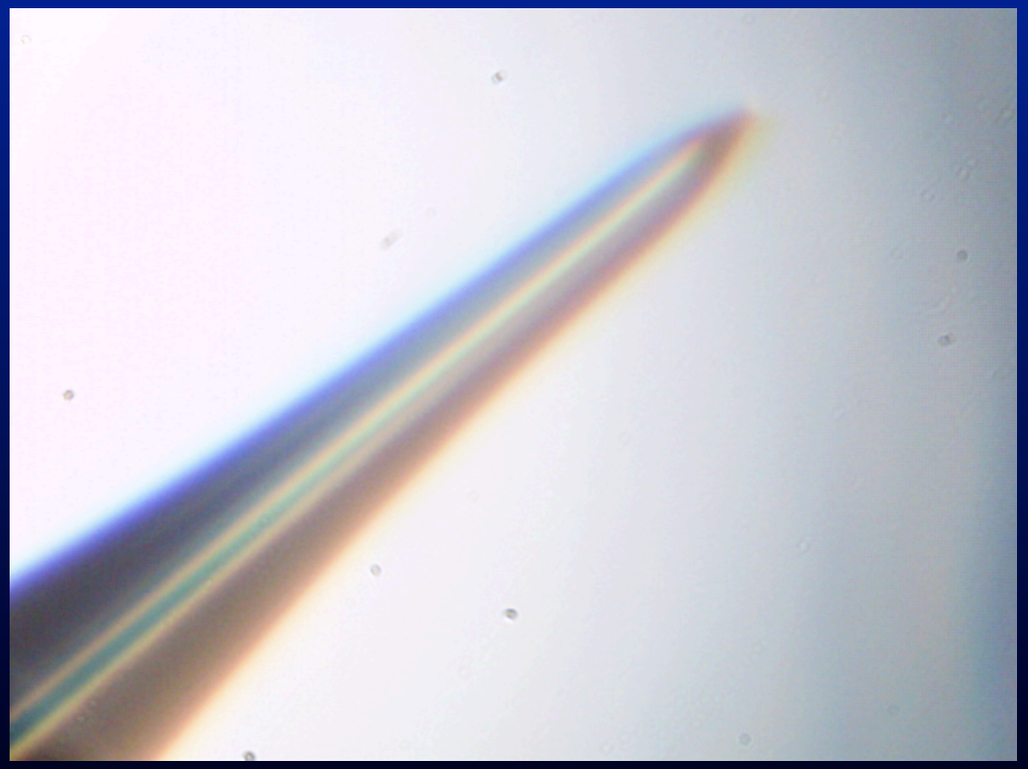
Update 1: Different capillary tube



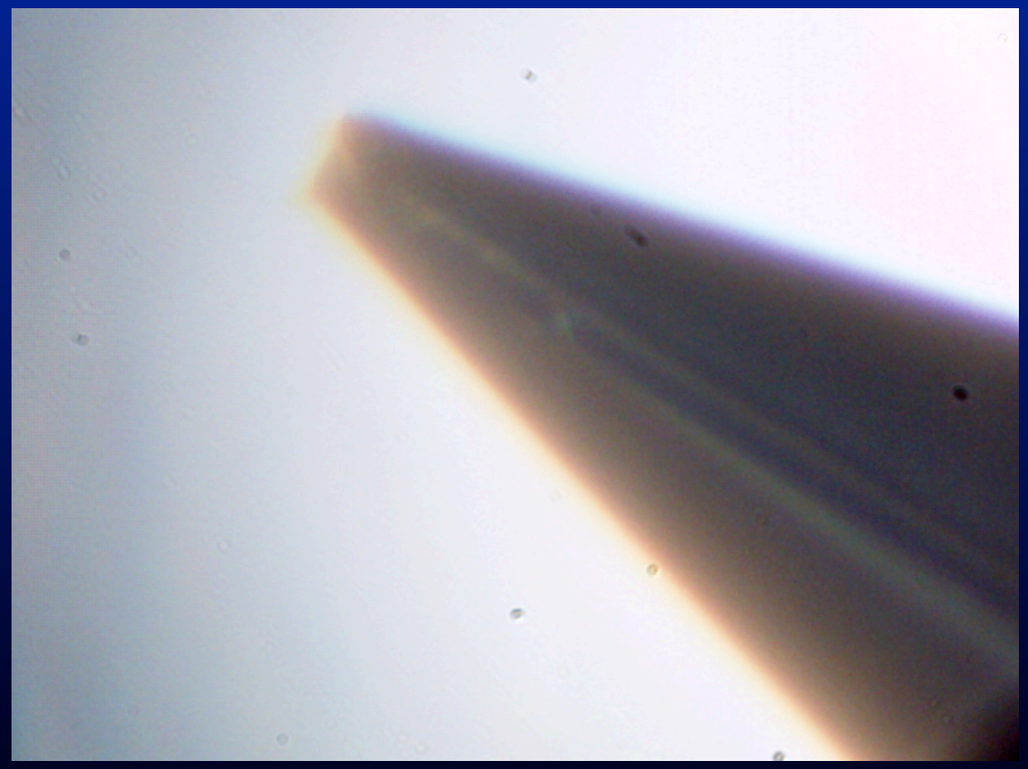
Problem: delicate tips would plug in days (solvent & debris)

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



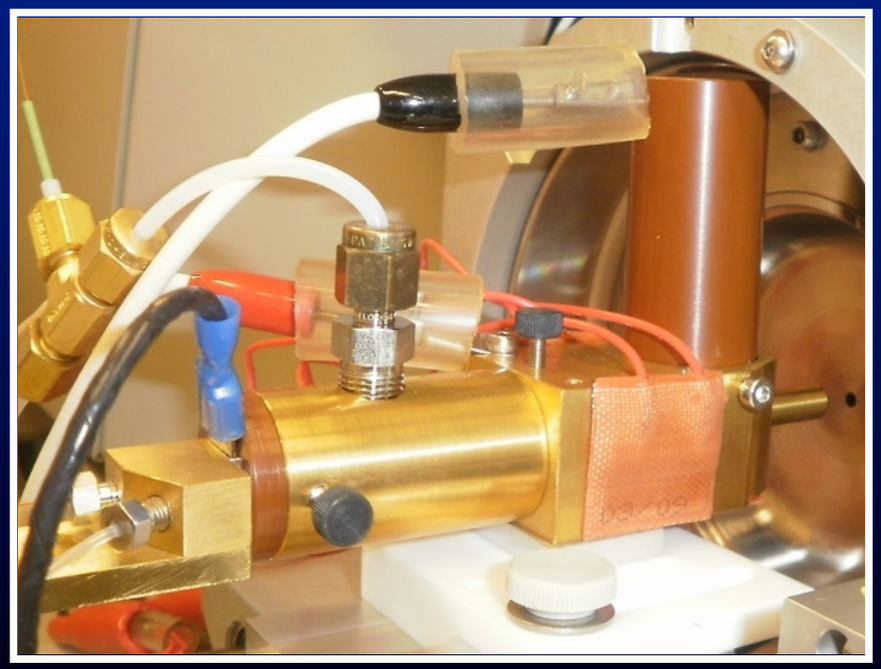
20 um const ID

Atmospheric Pressure - Electron Capture Dissociation
Update 2: Programmable Heater

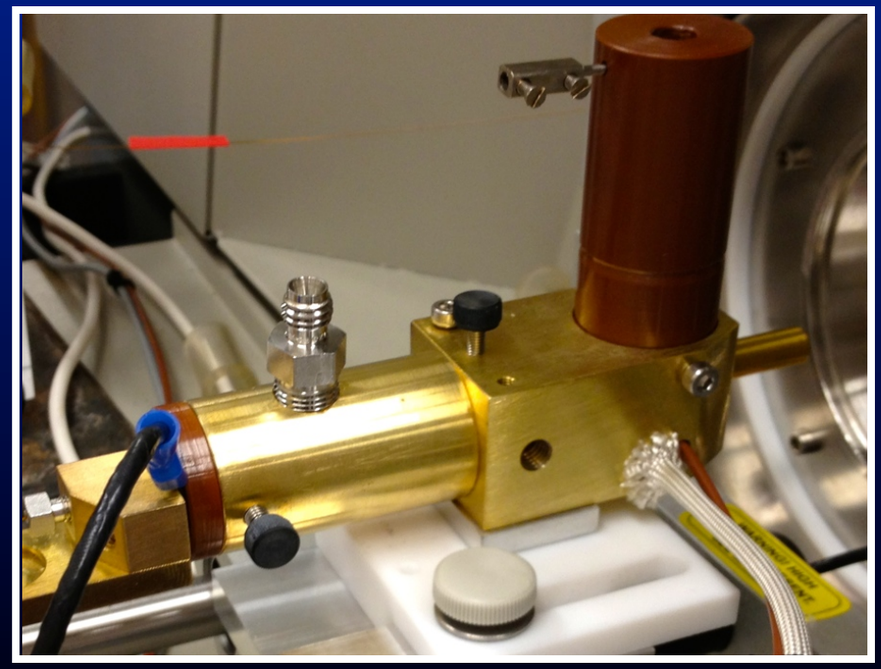
Problem: had to be turned off at night requiring subsequent daily
cleansing 1 hr bake outs
manual heater required attention to avoid burnout

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

Atmospheric Pressure - Electron Capture Dissociation
Update 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
Update 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

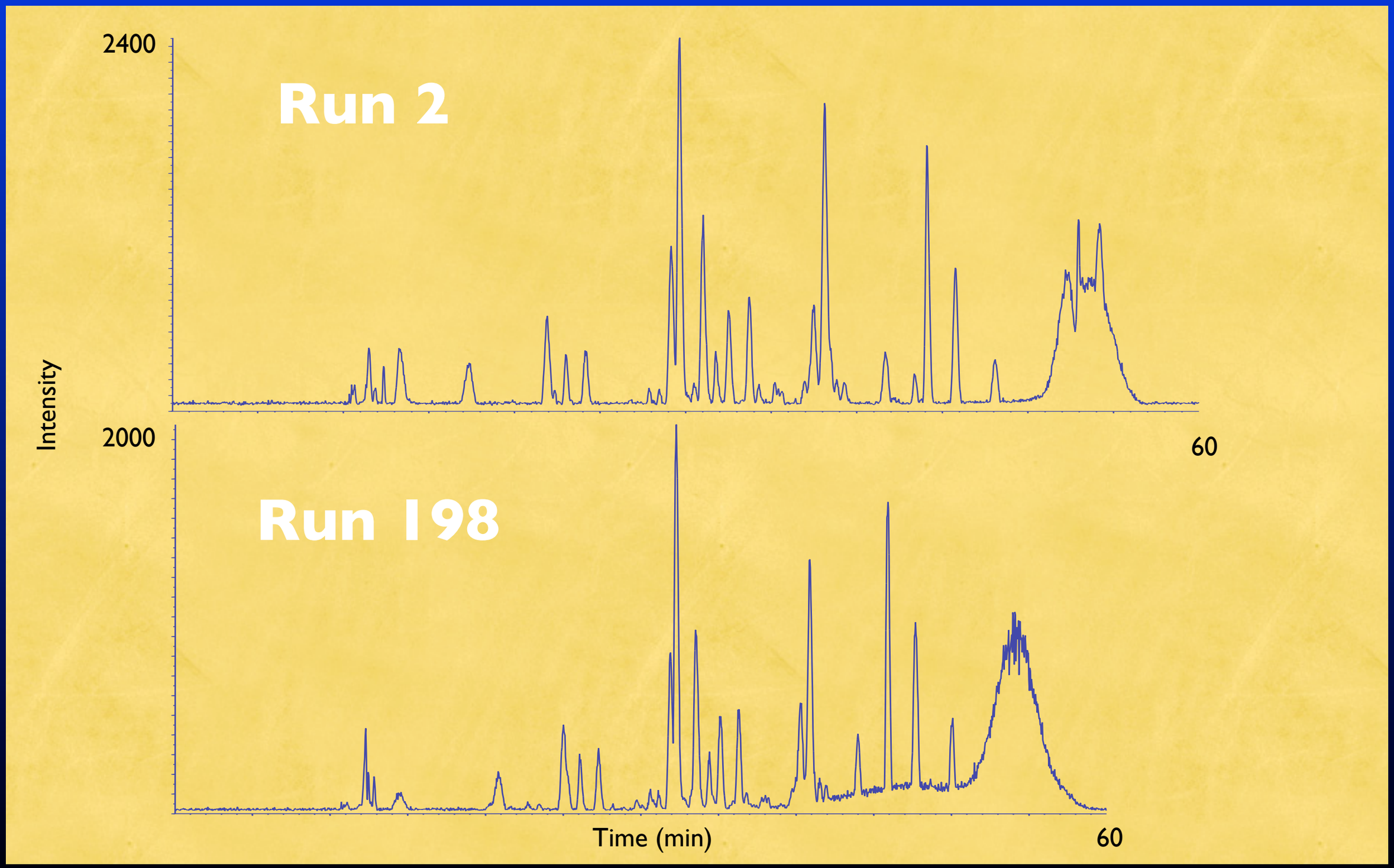
Atmospheric Pressure - Electron Capture Dissociation
Update 4: Reproducibilty

Problem: Unknown PM (performance/ maintaince) schedule

Solution: run it till it breaks

Result: ran continuously for 8 days (200 injections) with no loss of signal
stopped experiment early (could spend a year testing it)

Atmospheric Pressure - Electron Capture Dissociation
Update 4: Reproducibilty



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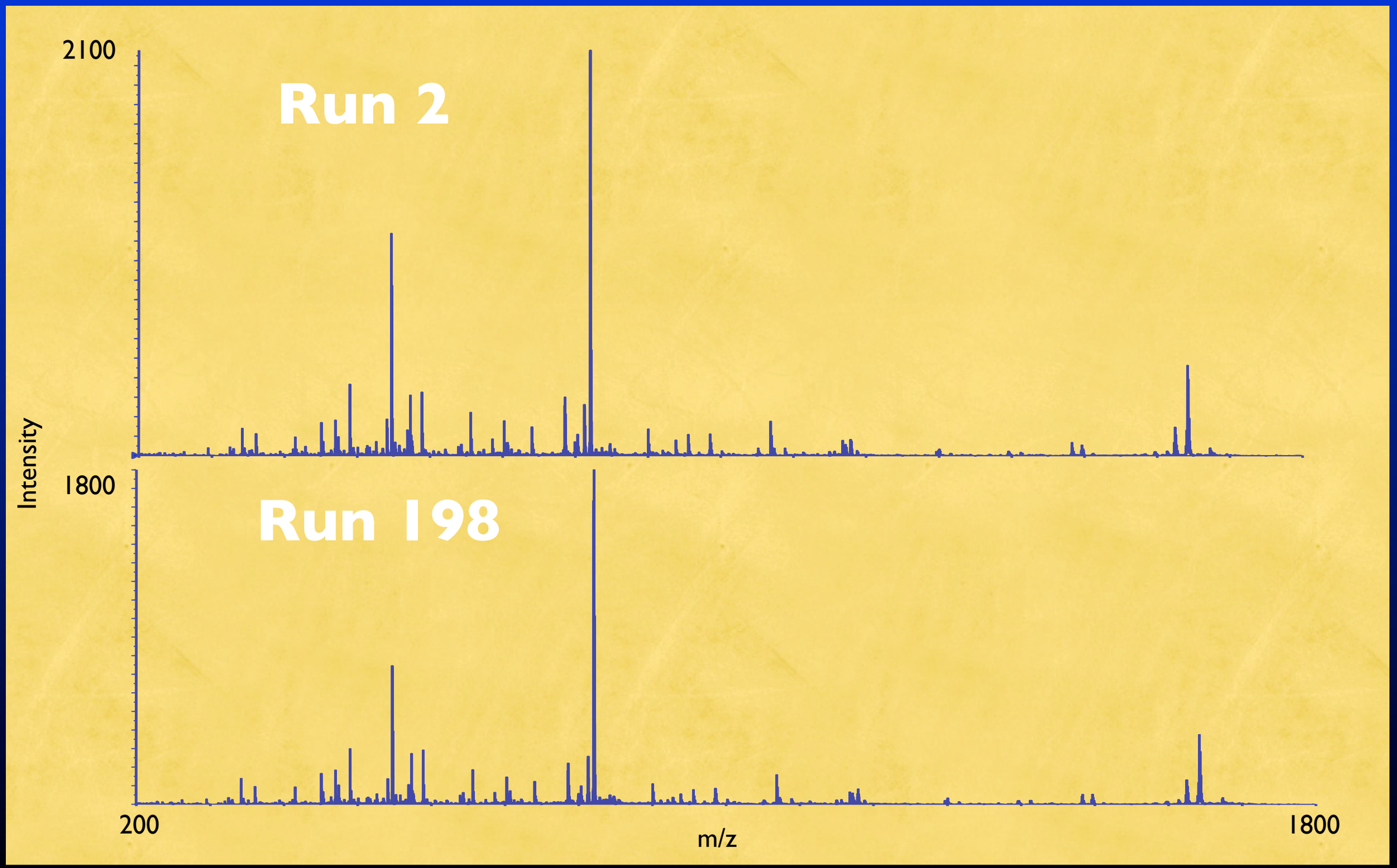


Atmospheric Pressure - Electron Capture Dissociation
Update 5: Hydrogen Deuterium exchange evaluation

Why: Structural proteomics

Current methods: enzymatic digestion (limited sequence coverage)
ETD (electron transfer dissociation), new instrument

Desire: Add HDX capibility onto our instruments

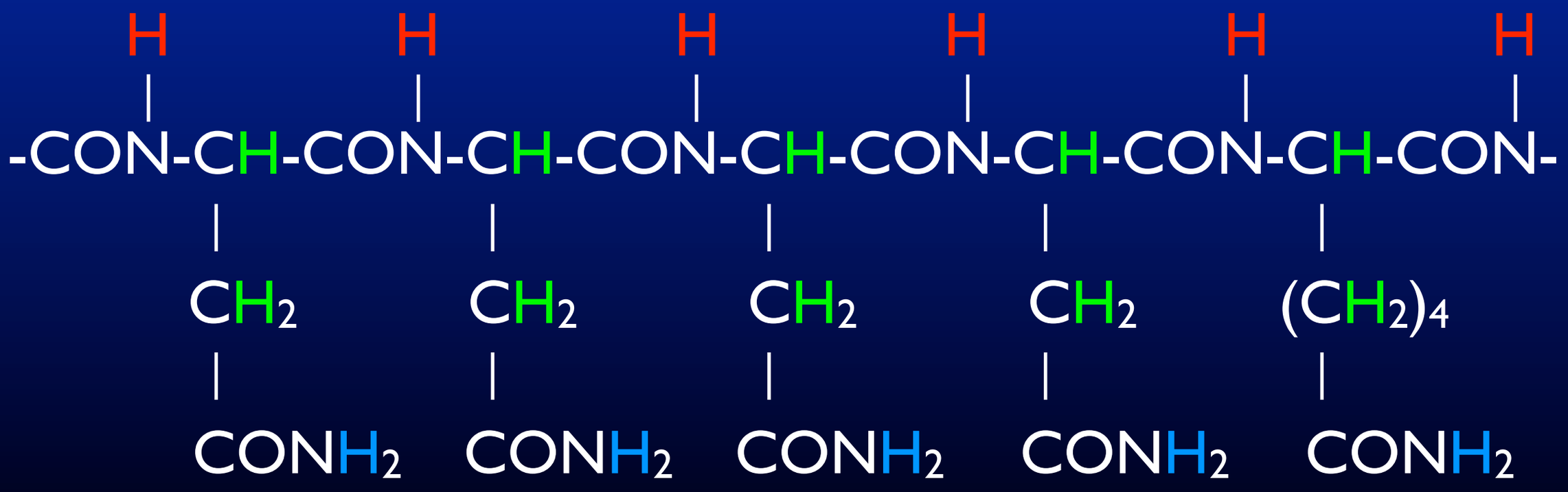
Problems: HDX doesn't work with CID

need minimal scrambling of deuteriums

Atmospheric Pressure - Electron Capture Dissociation

Update 5: Hydrogen Deuterium exchange evaluation

Green: Do not exchange
Blue: Exchange too fast
Red: Exchange can be measured



Atmospheric Pressure - Electron Capture Dissociation
Update 5: Hydrogen Deuterium exchange evaluation

Need model peptide to study deuterium scrambling

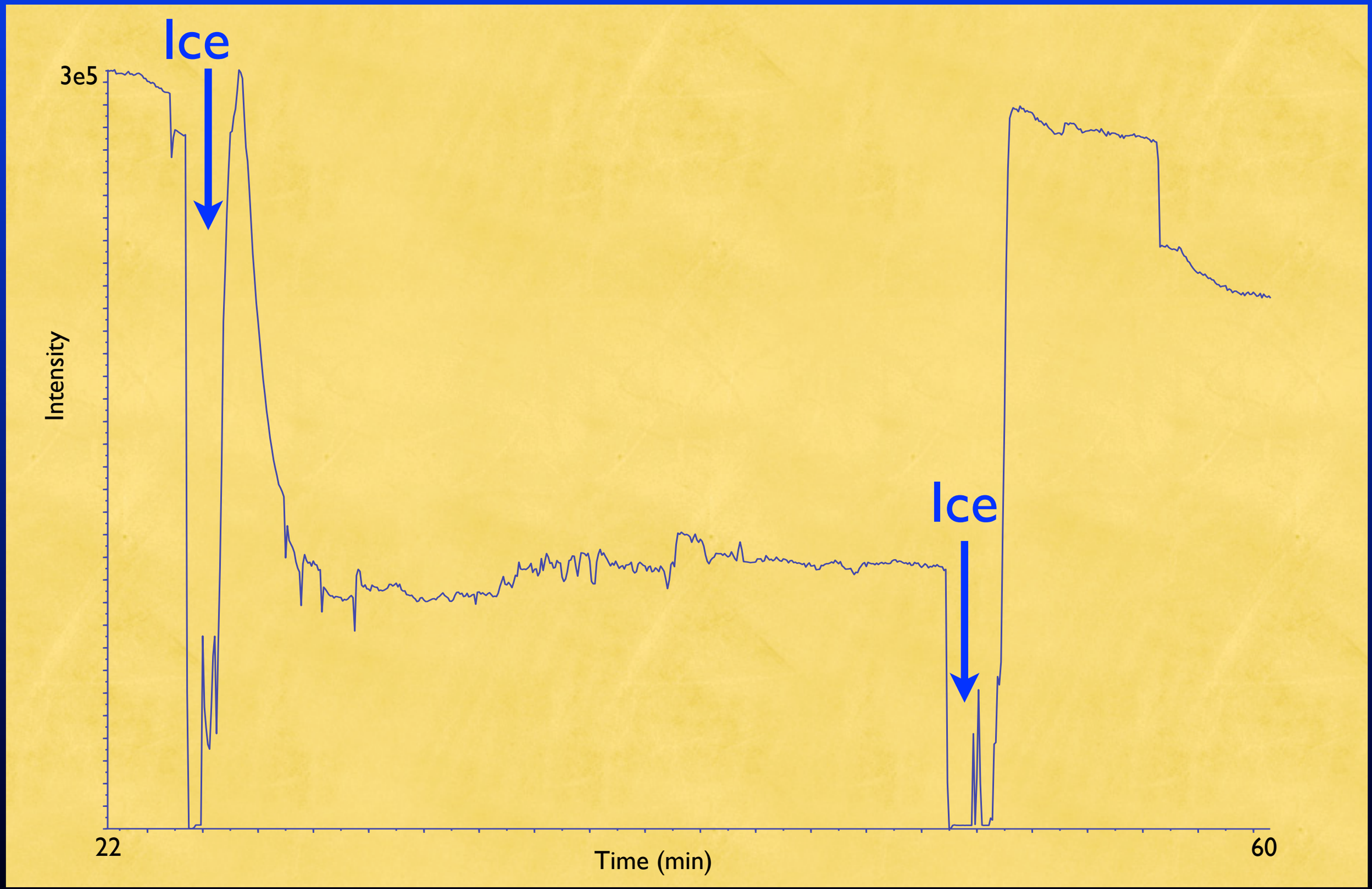
H H H H H H H I I K I I K

by design c-terminal is preferentially labelled, n-terminal is not

Steps:
- mix peptide in acidified D_20
- mix with acidified $MeOH/H_2O$ for Hydrogen exchange
- measure isotope ratios via ECD MS

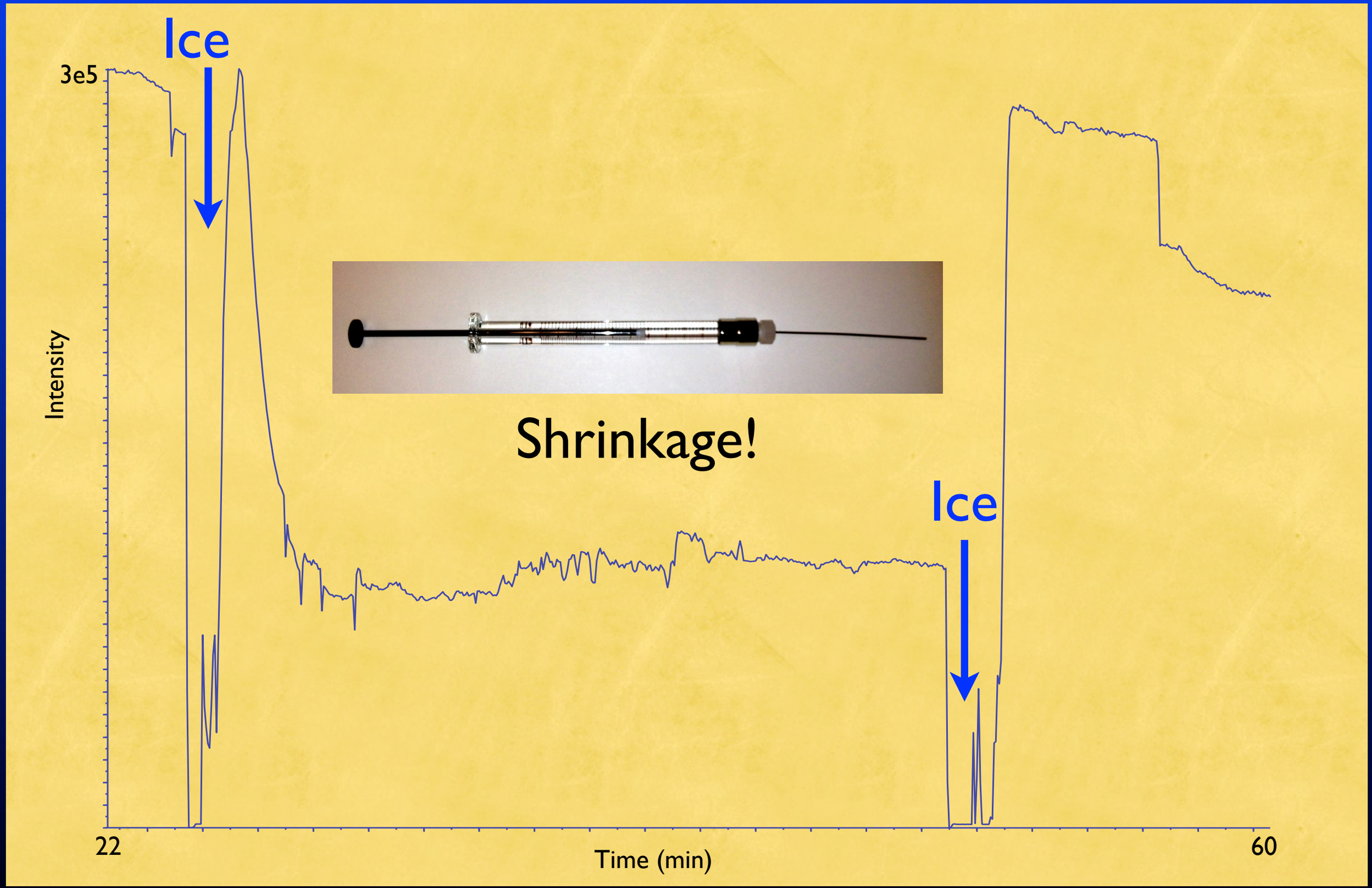
Calls for acidic and cold conditions to slow back reaction down

Atmospheric Pressure - Electron Capture Dissociation
Update 5: HDX - Syringe Cooling



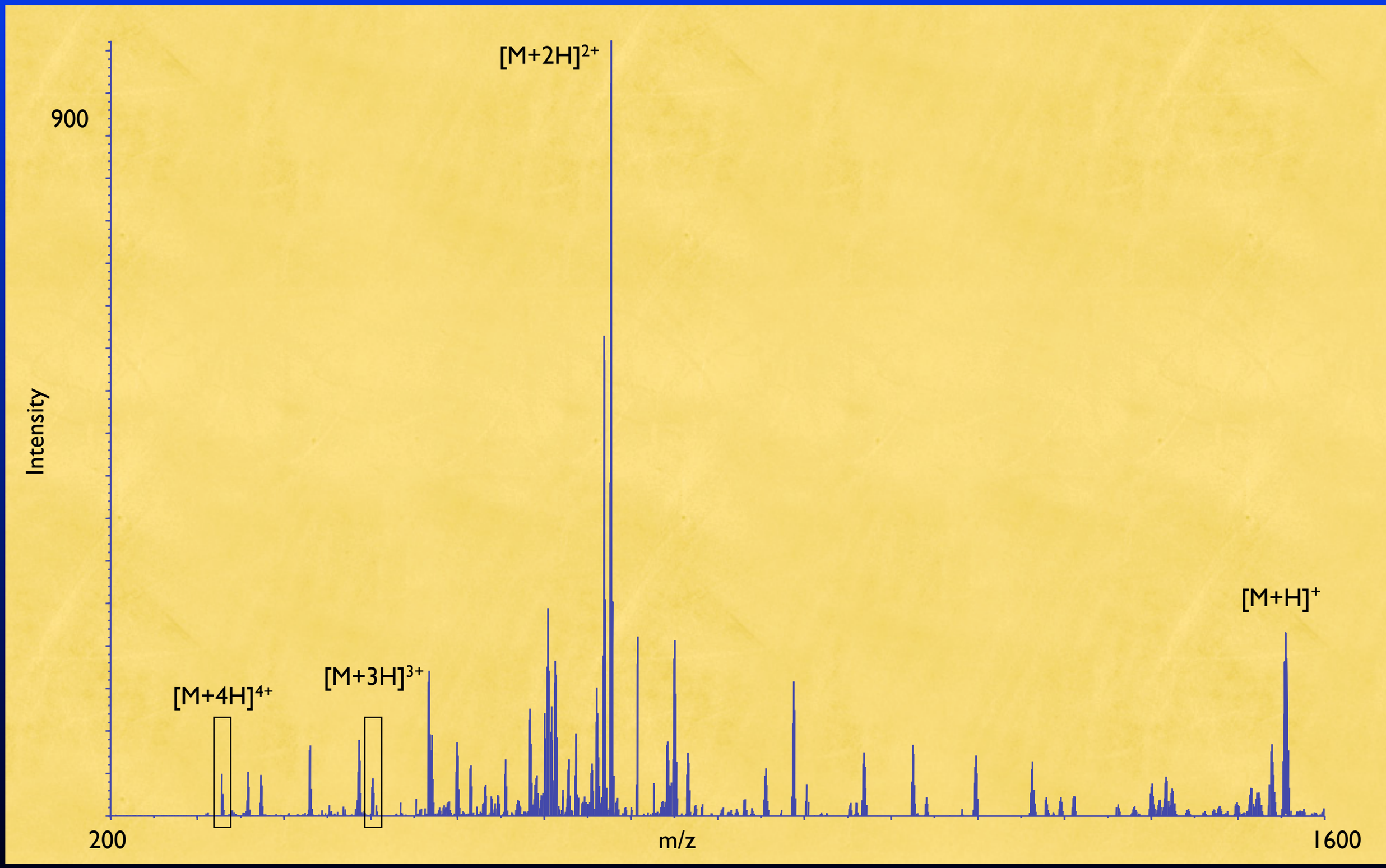
TIC P1

Atmospheric Pressure - Electron Capture Dissociation
Update 5: HDX - Syringe Cooling



TIC P1

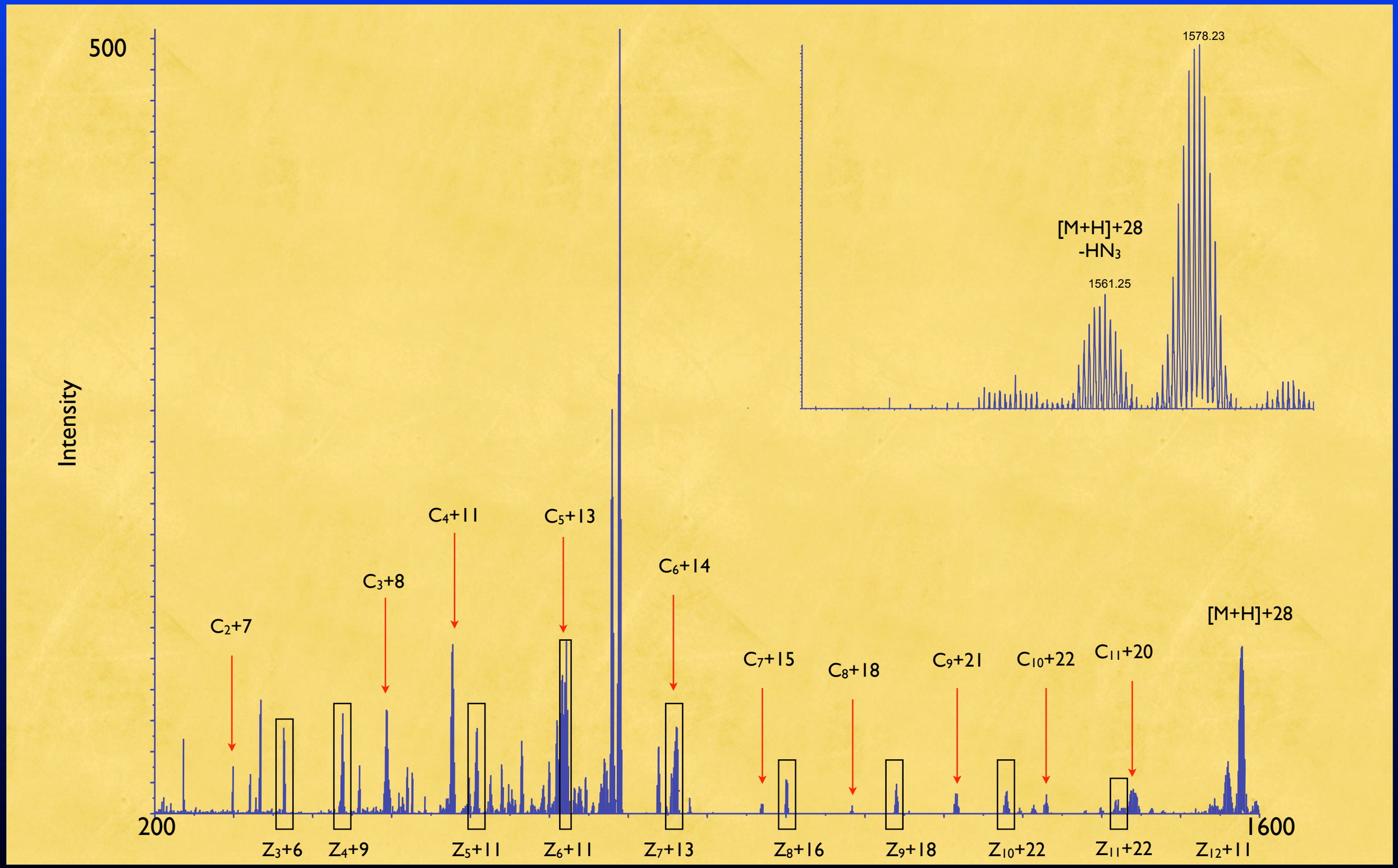
Atmospheric Pressure - Electron Capture Dissociation
Update 4: HDX



ECD P1 8 um

Atmospheric Pressure - Electron Capture Dissociation

Update 4: HDX of P1 in D_2O



ECD P1 8 um

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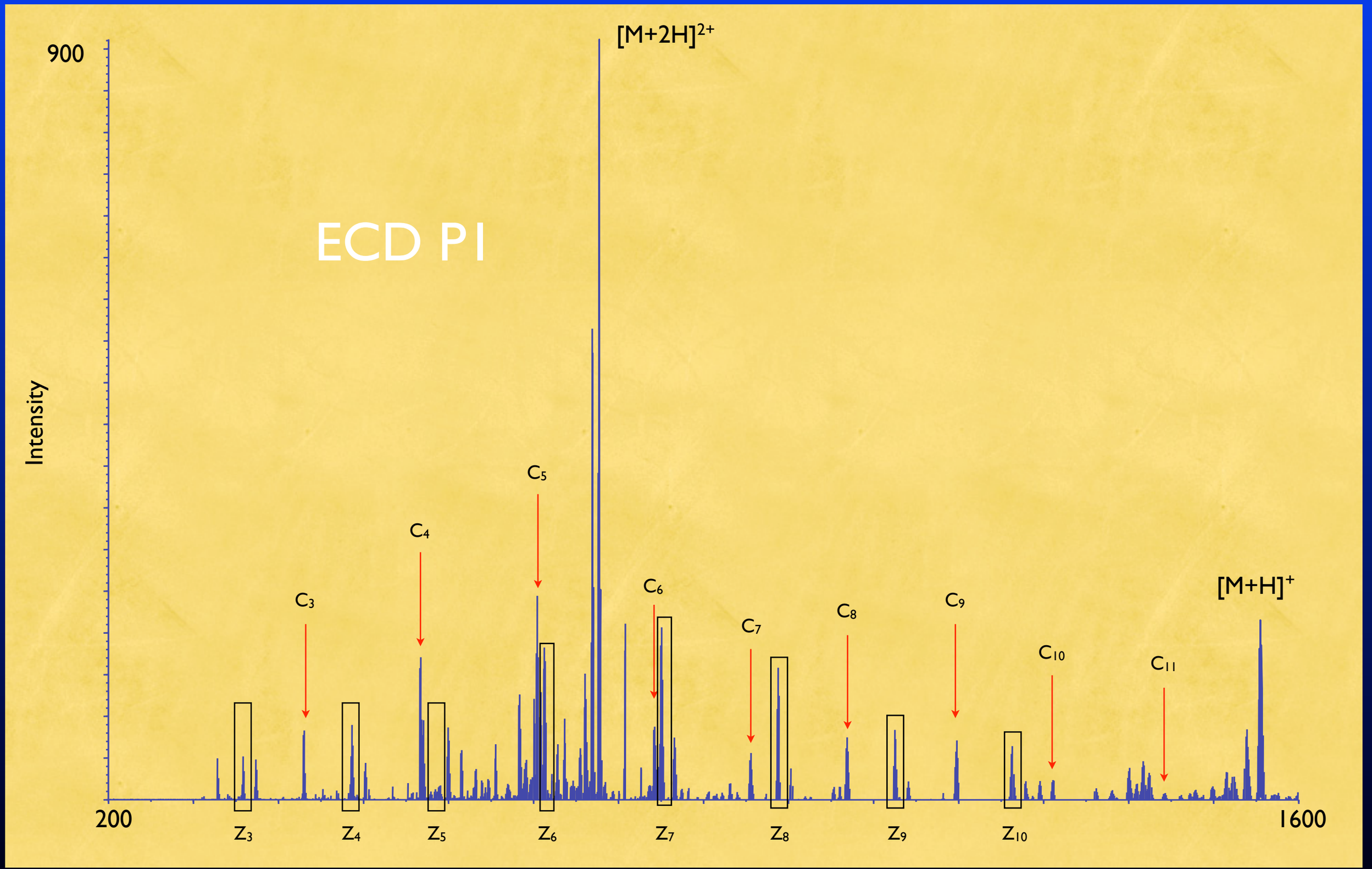

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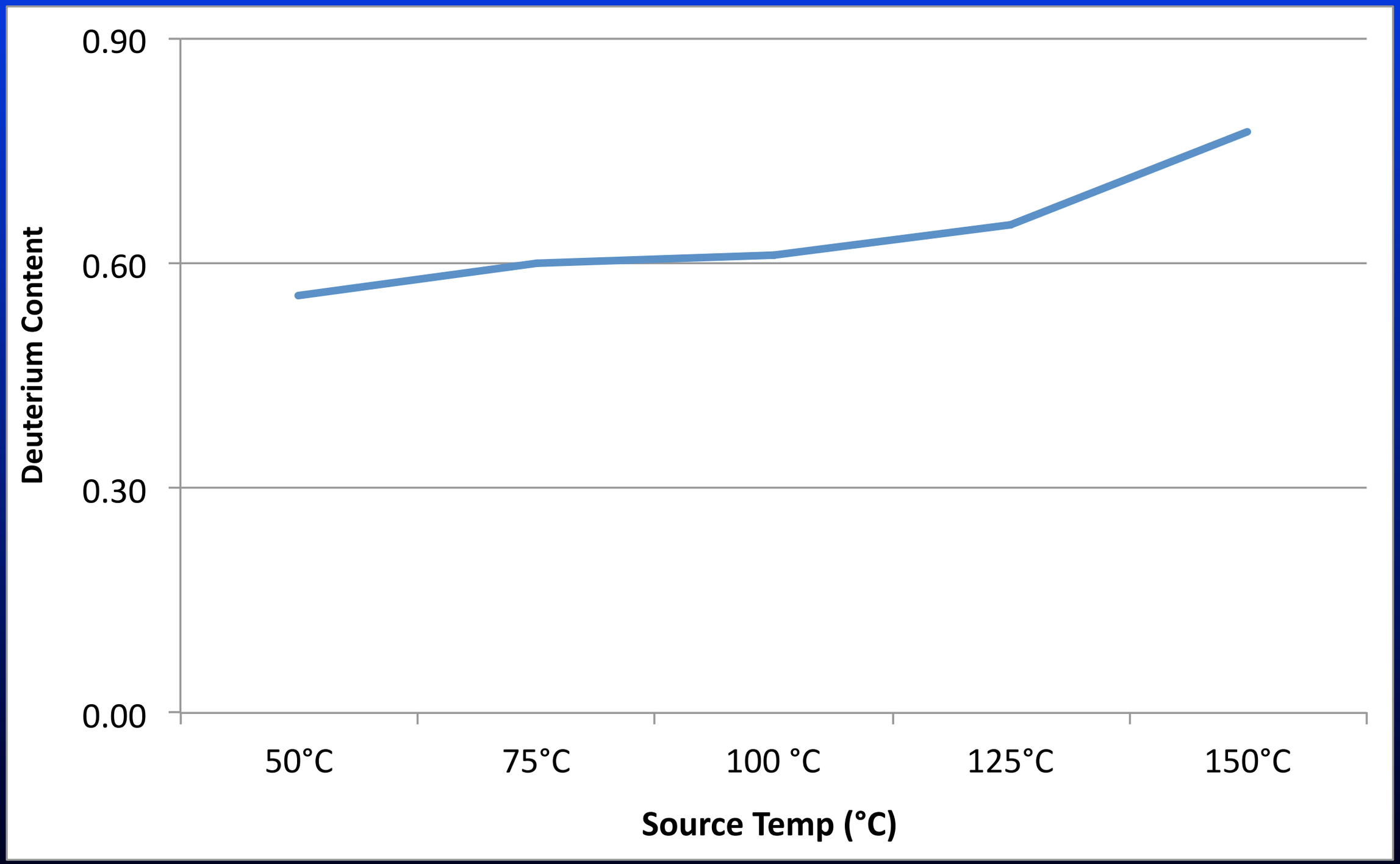


Deuterium scrambling with temperature (C3 ion)

Coming up.....

Finish HX experiments (Dec 31)
reduce deuterium scrambling

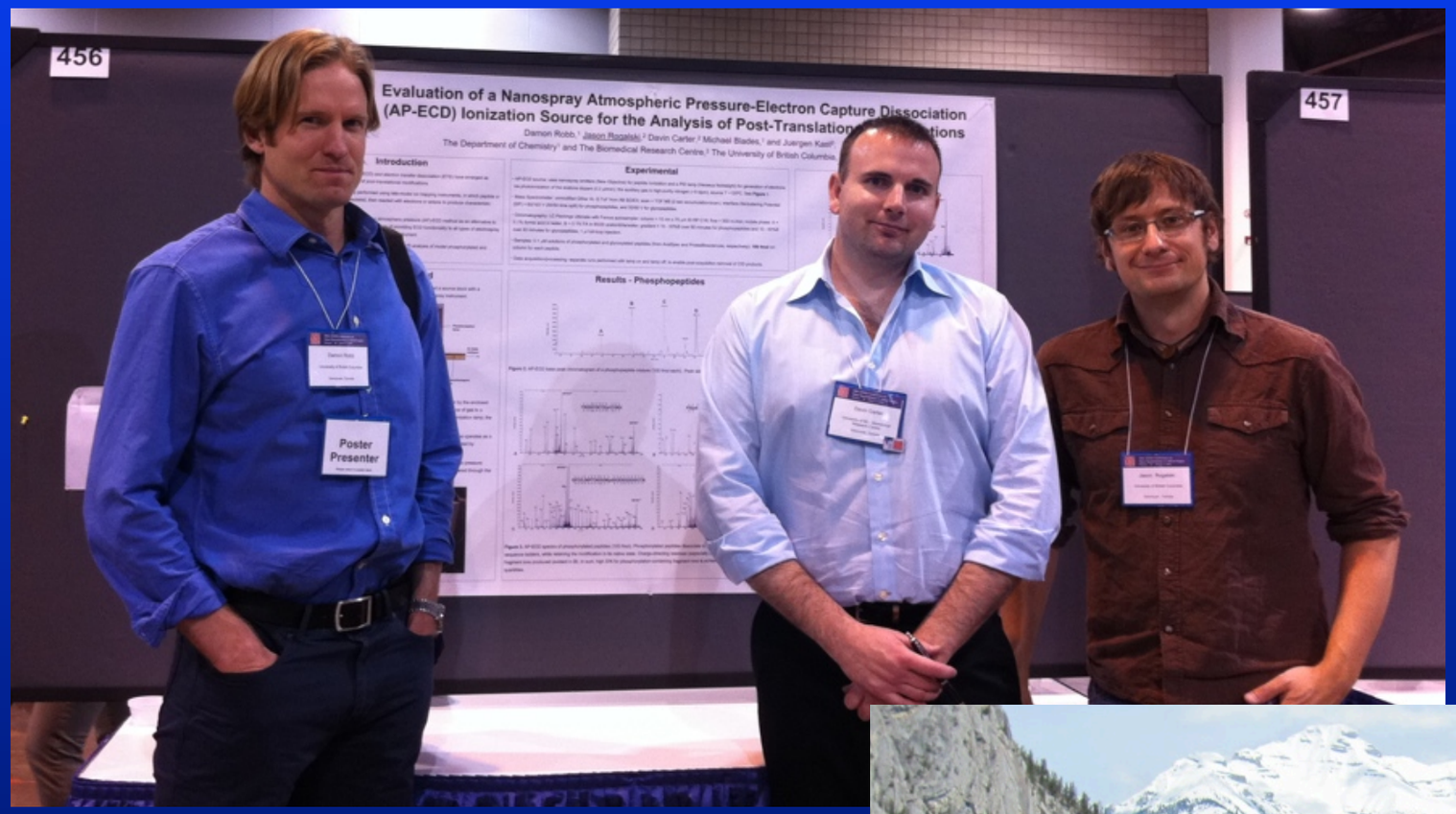
Survey of PTMs (Jan 31)

ECD combined with CID screening

Histones --> chrom 6

Acknowledgements

Kast Lab

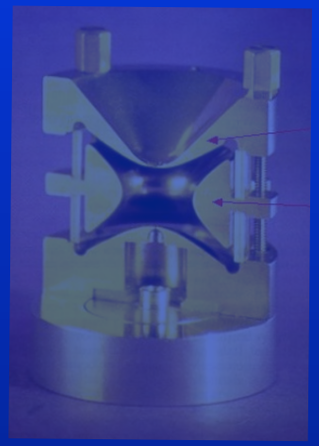


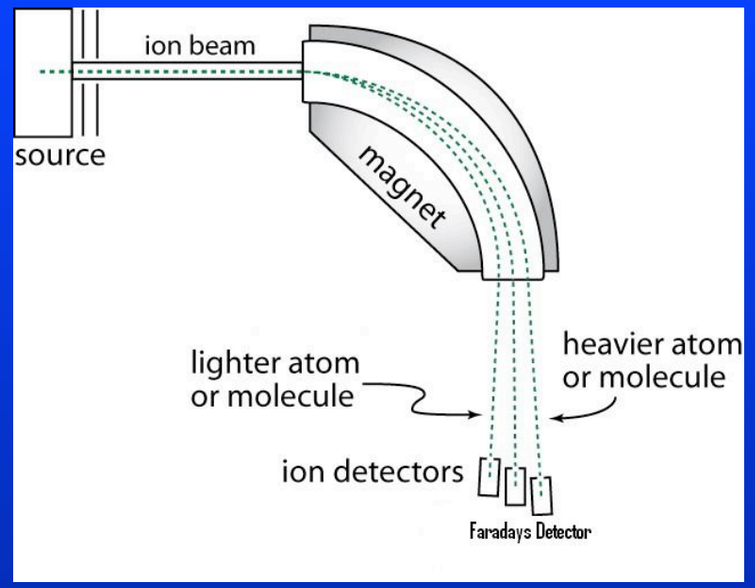
Thanks.....

a place of mind

Mass Spectrometry
Different types

Magnetic Sector

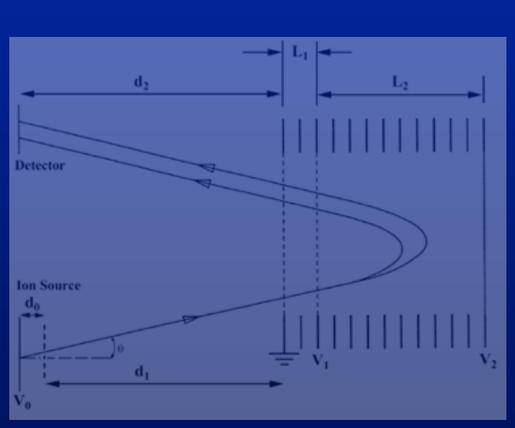




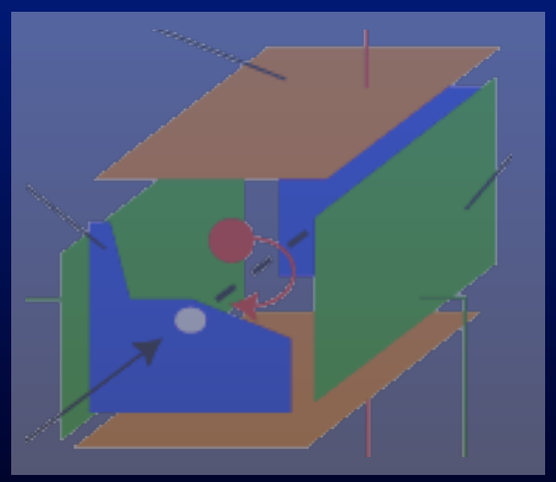
Ion Trap

Quadrupole

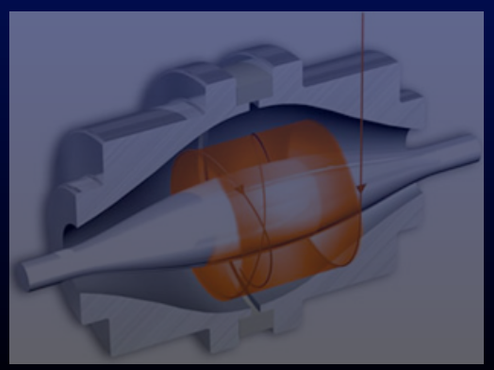
Time of Flight



Ion Cyclotron Resonance



Orbitrap



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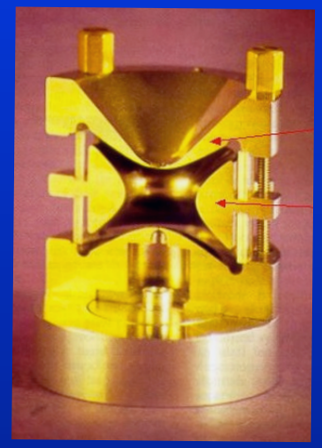

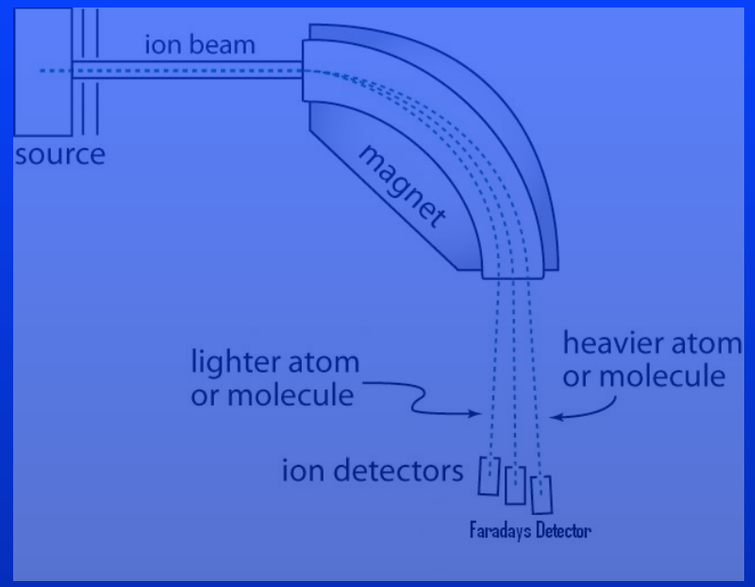

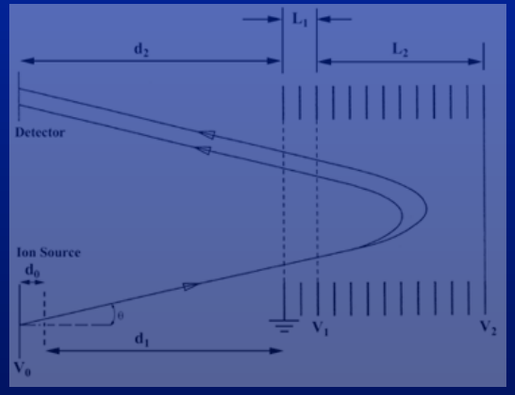

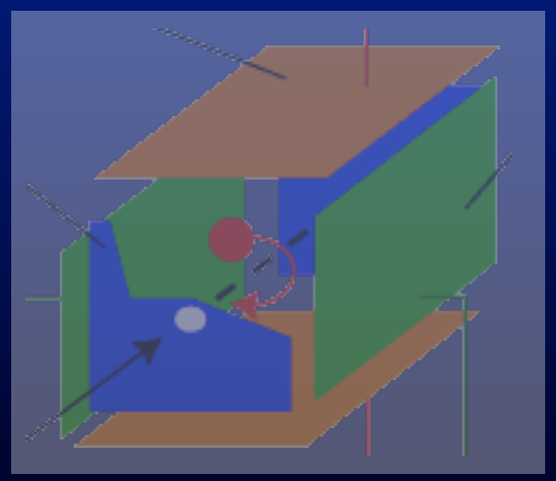

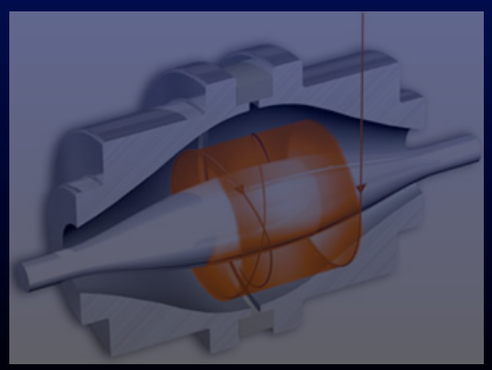

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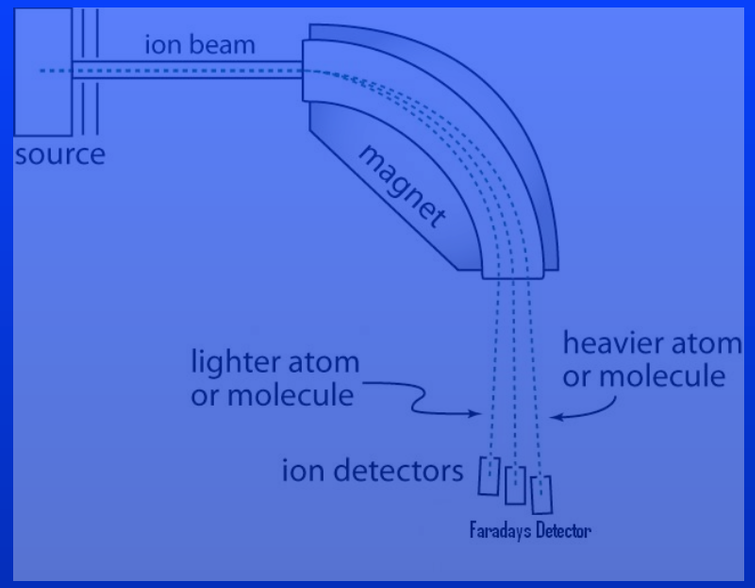

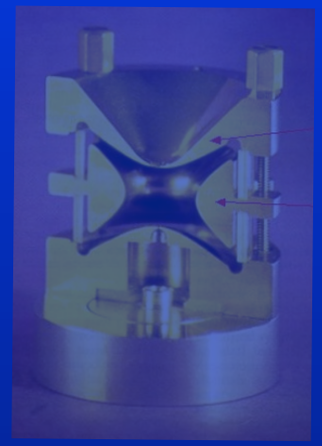

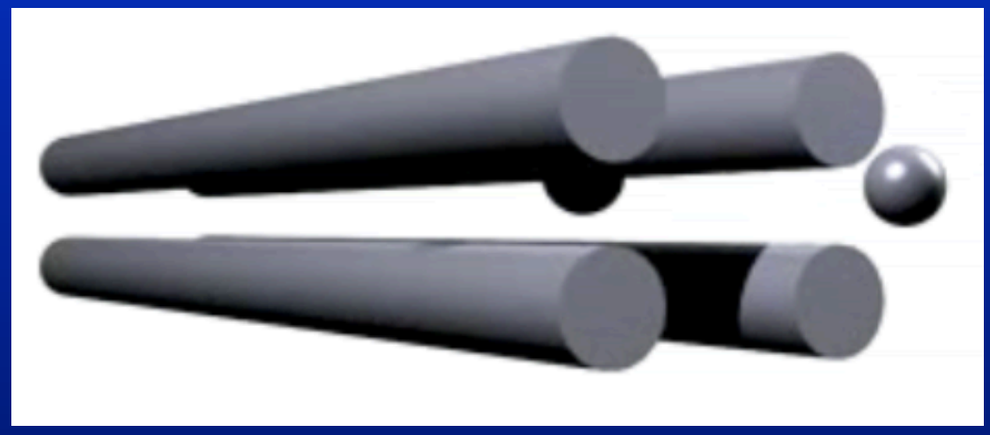

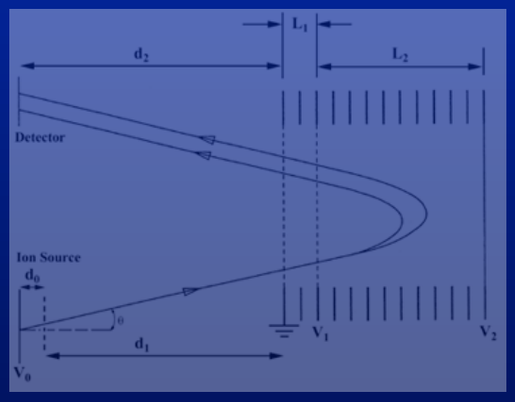

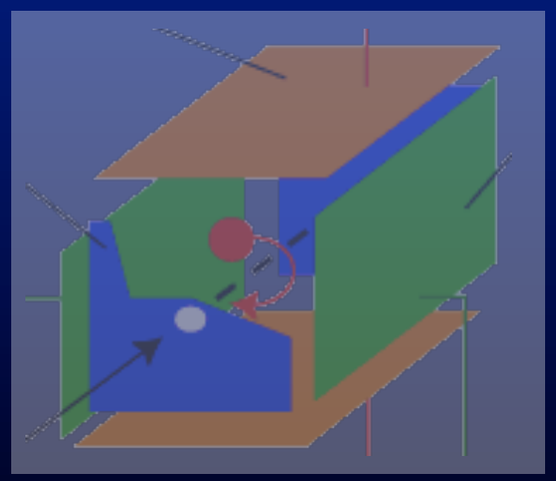

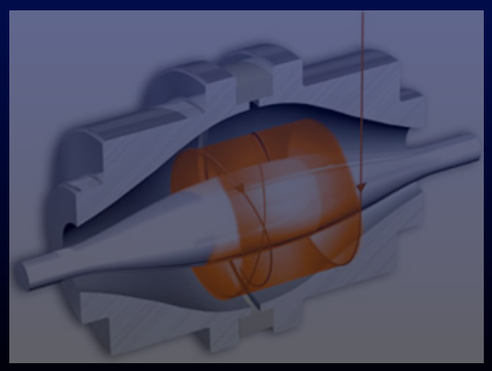

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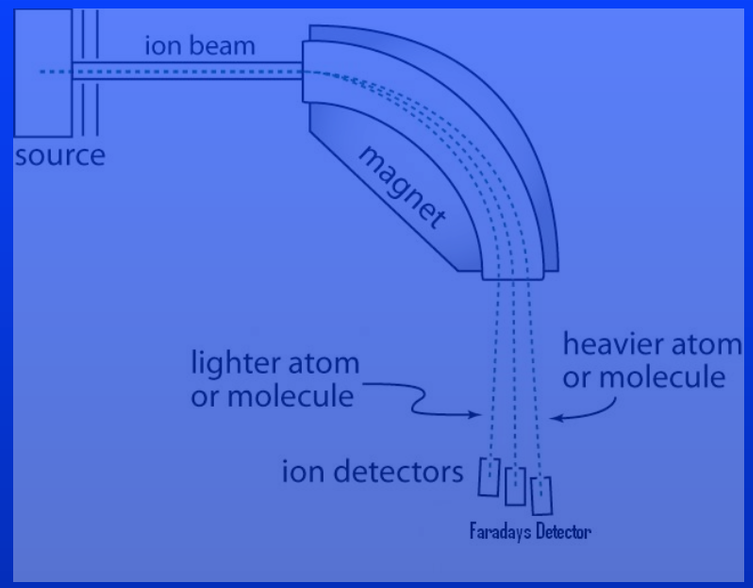

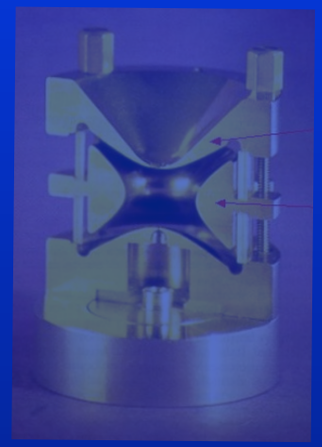

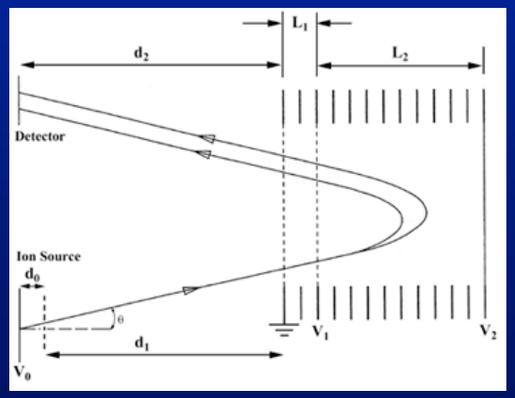

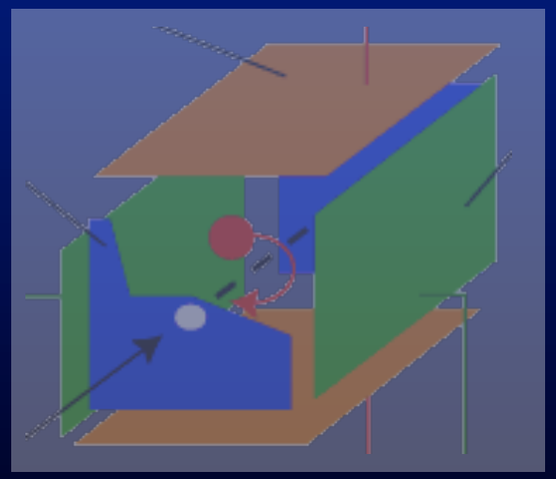

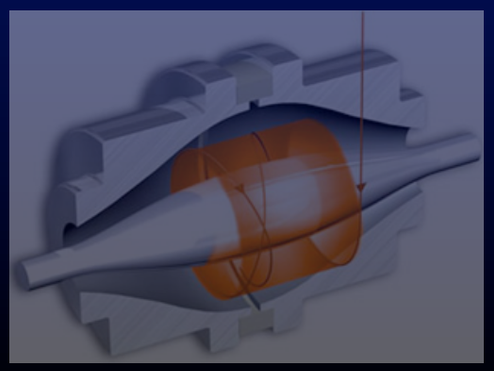

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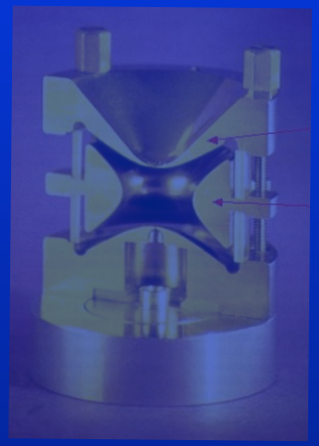

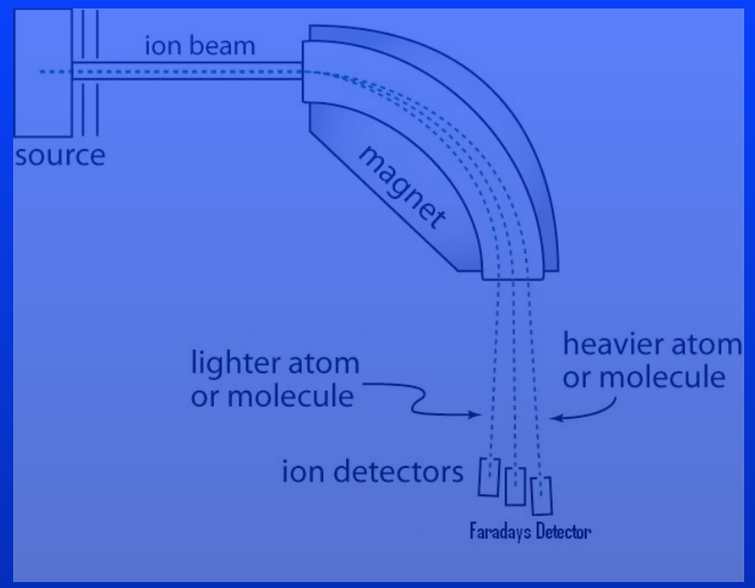

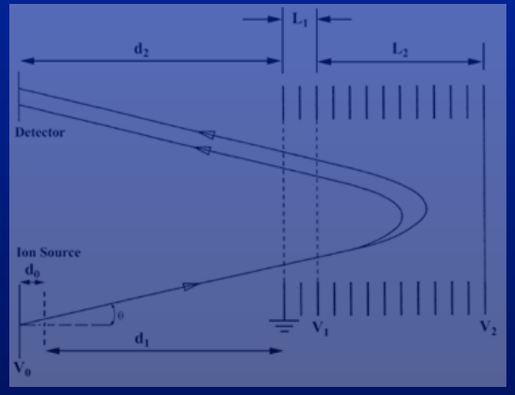

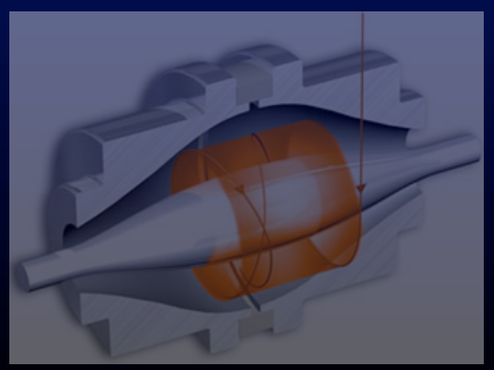

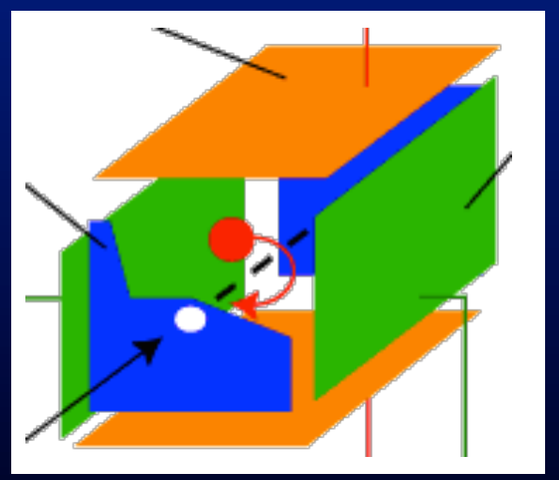

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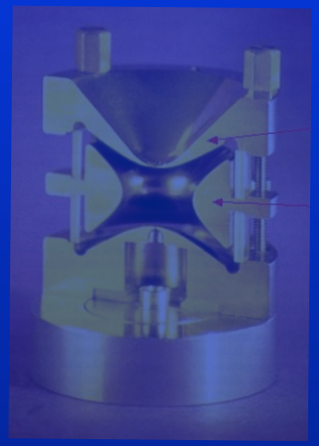
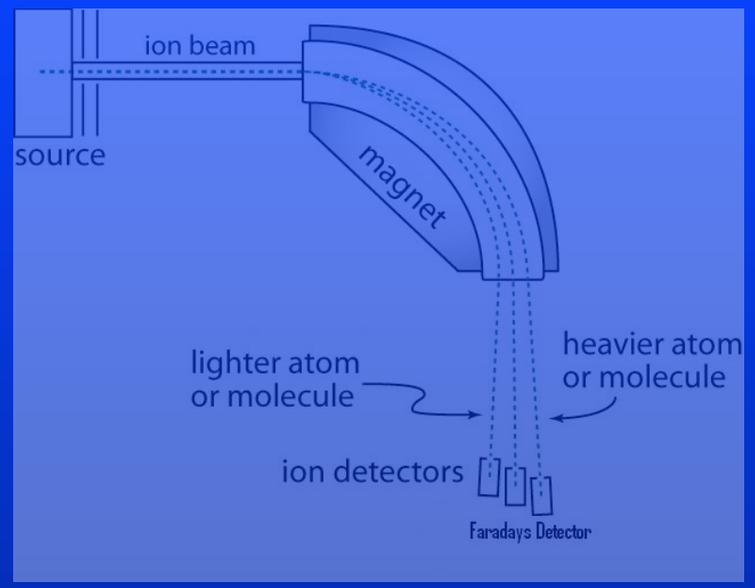
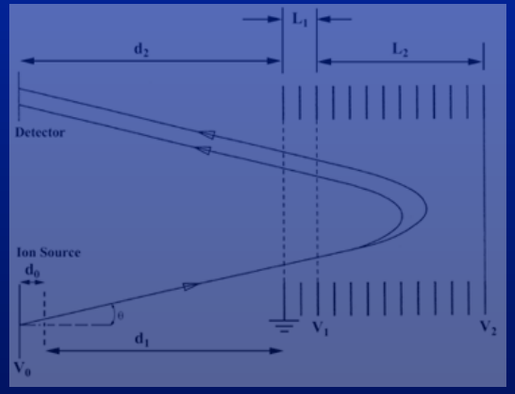
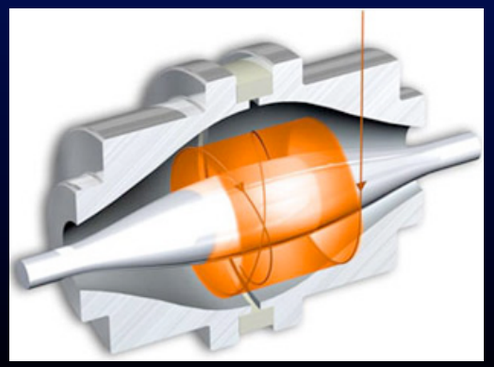
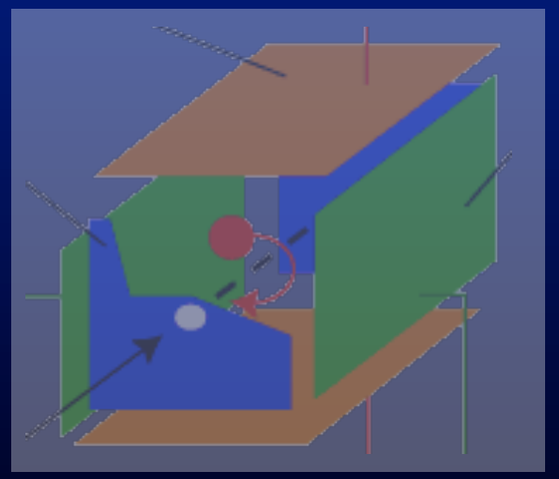


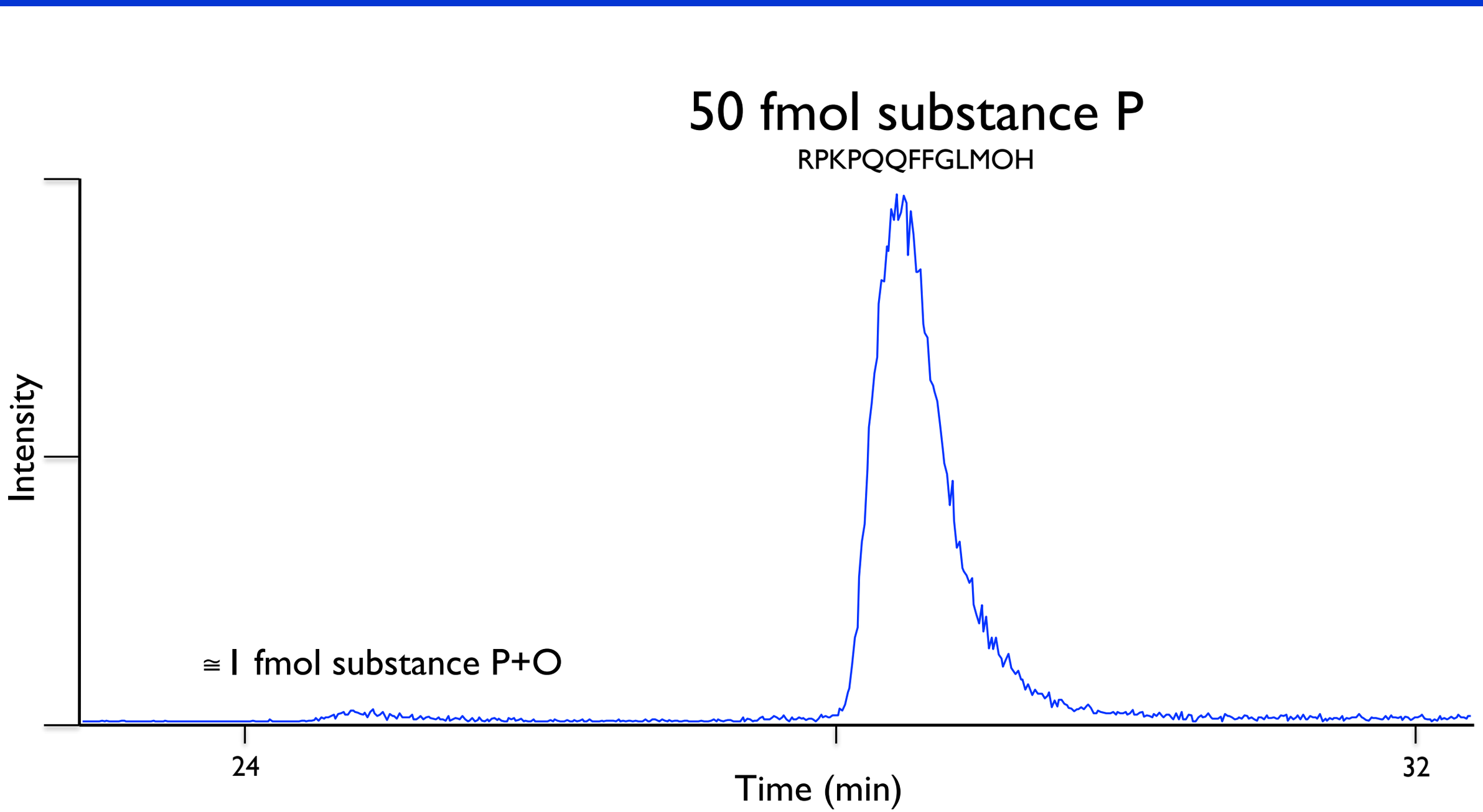




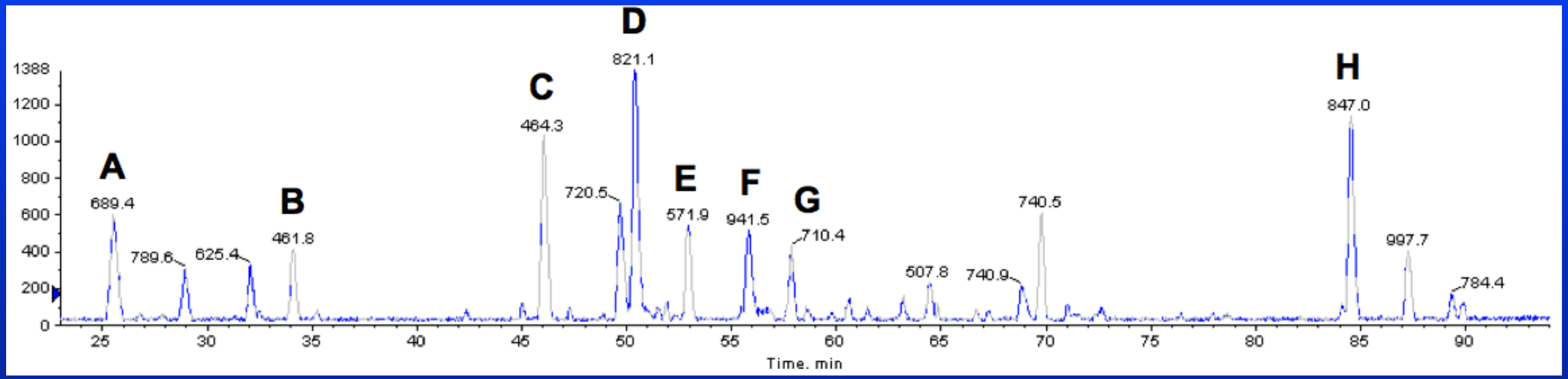


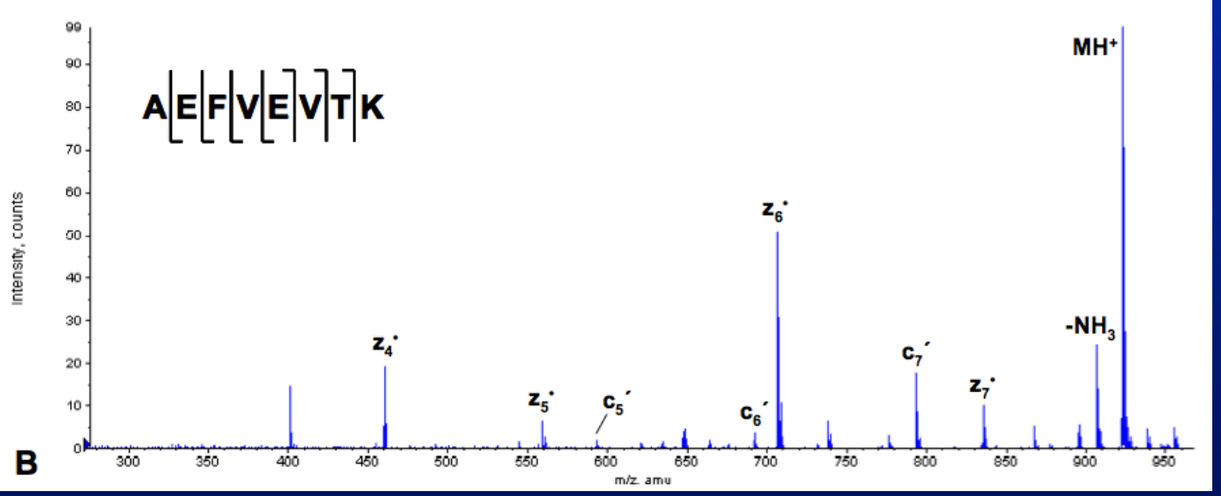


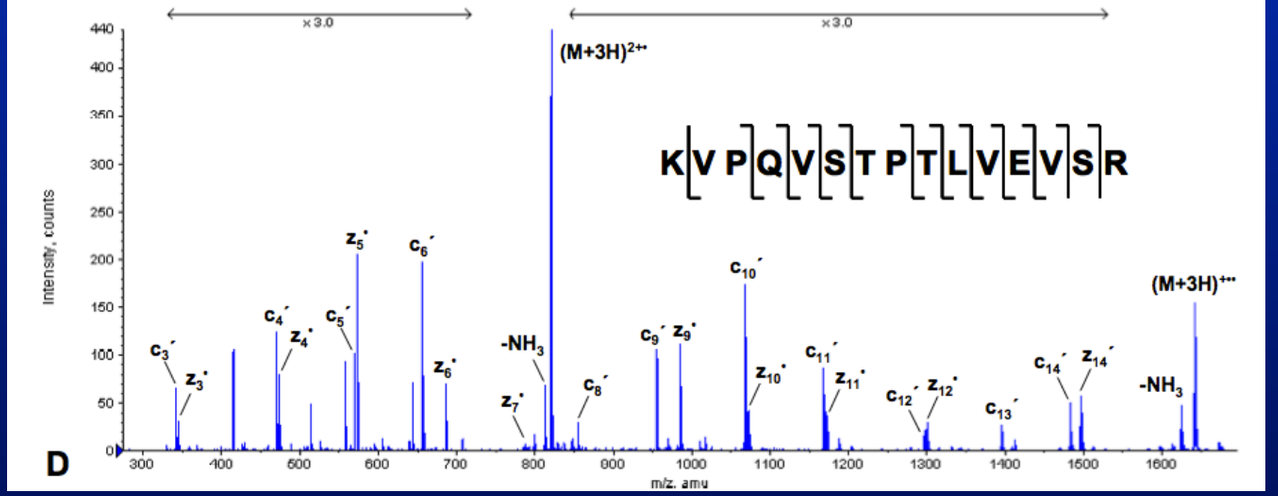
Atmospheric Pressure - Electron Capture Dissociation
Results - Sensitivity

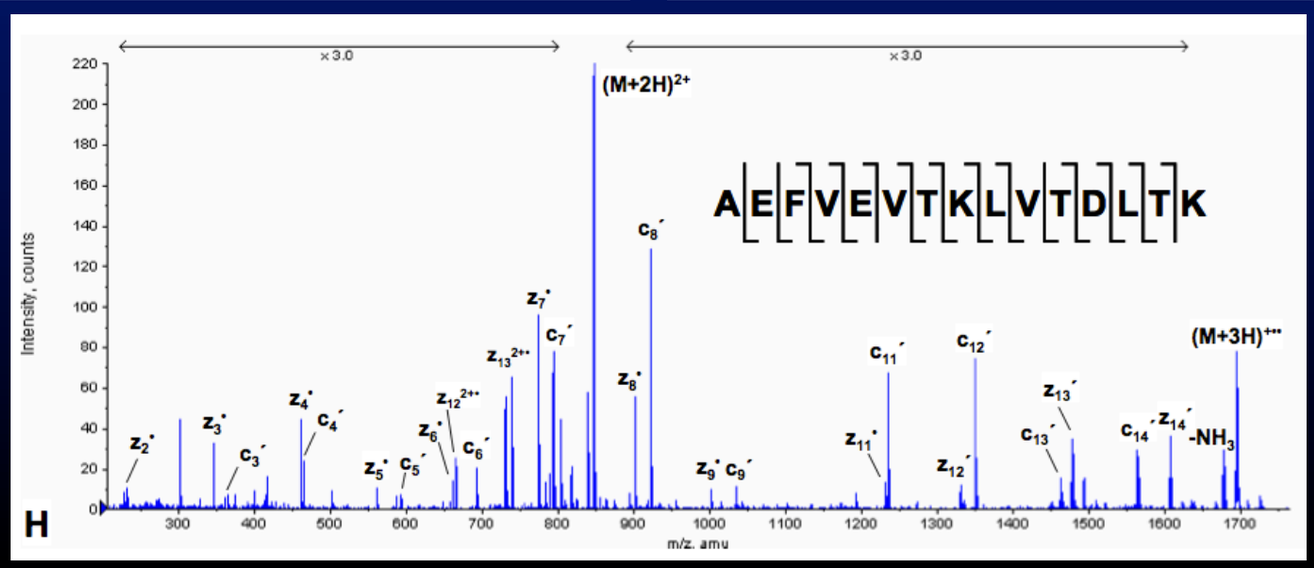


Atmospheric Pressure - Electron Capture Dissociation
Results - Mixtures



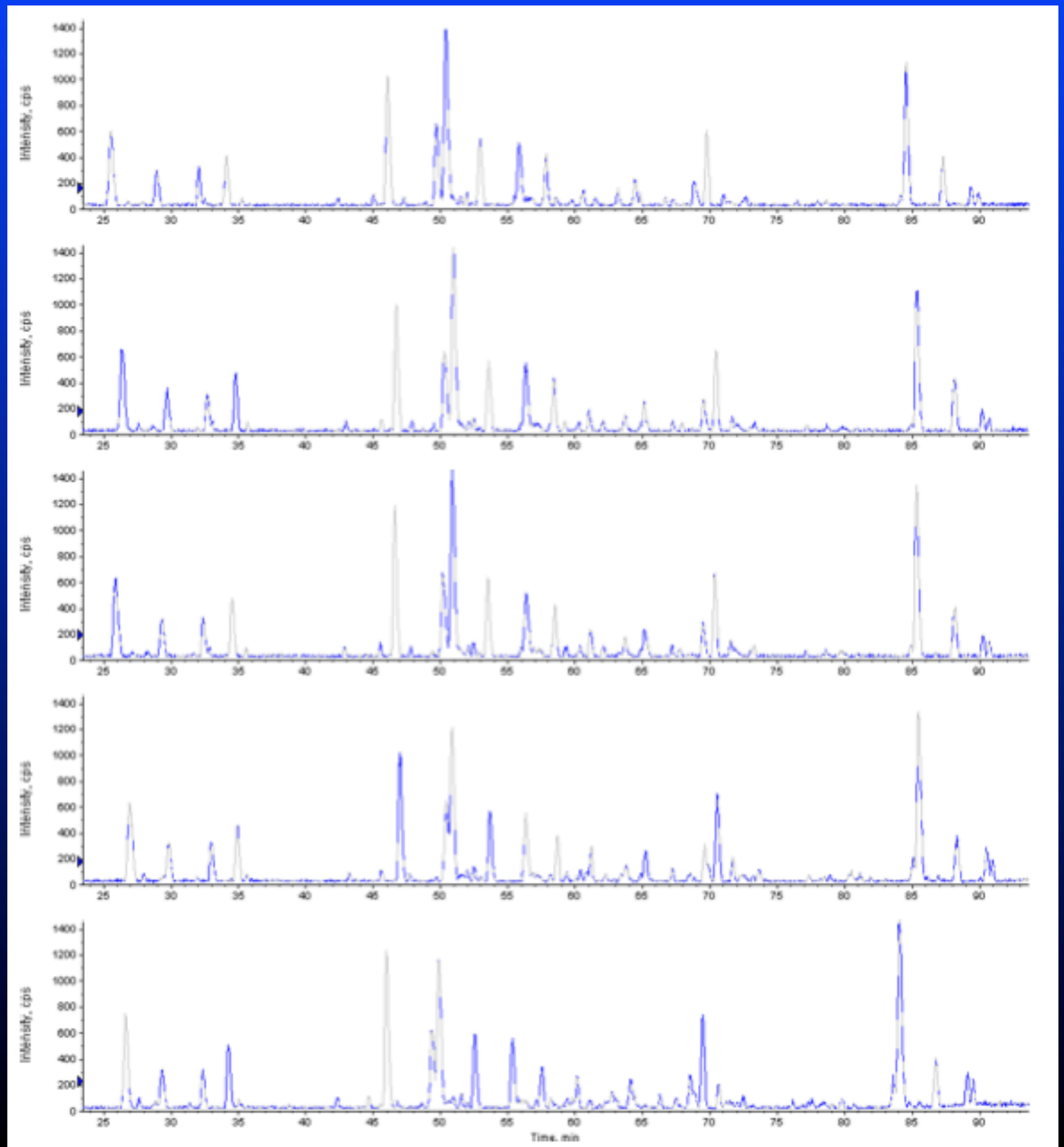




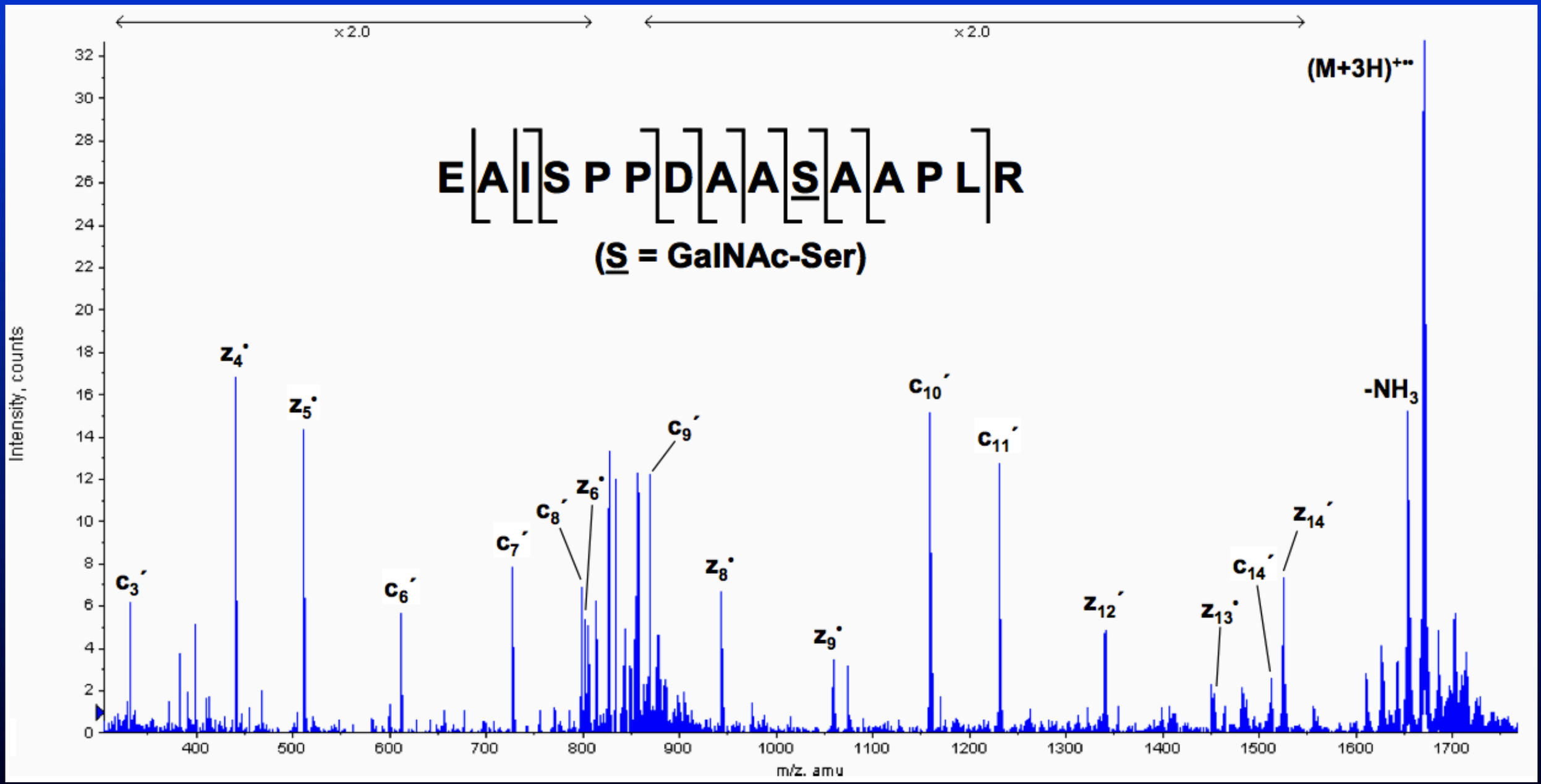


Atmospheric Pressure - Electron Capture Dissociation

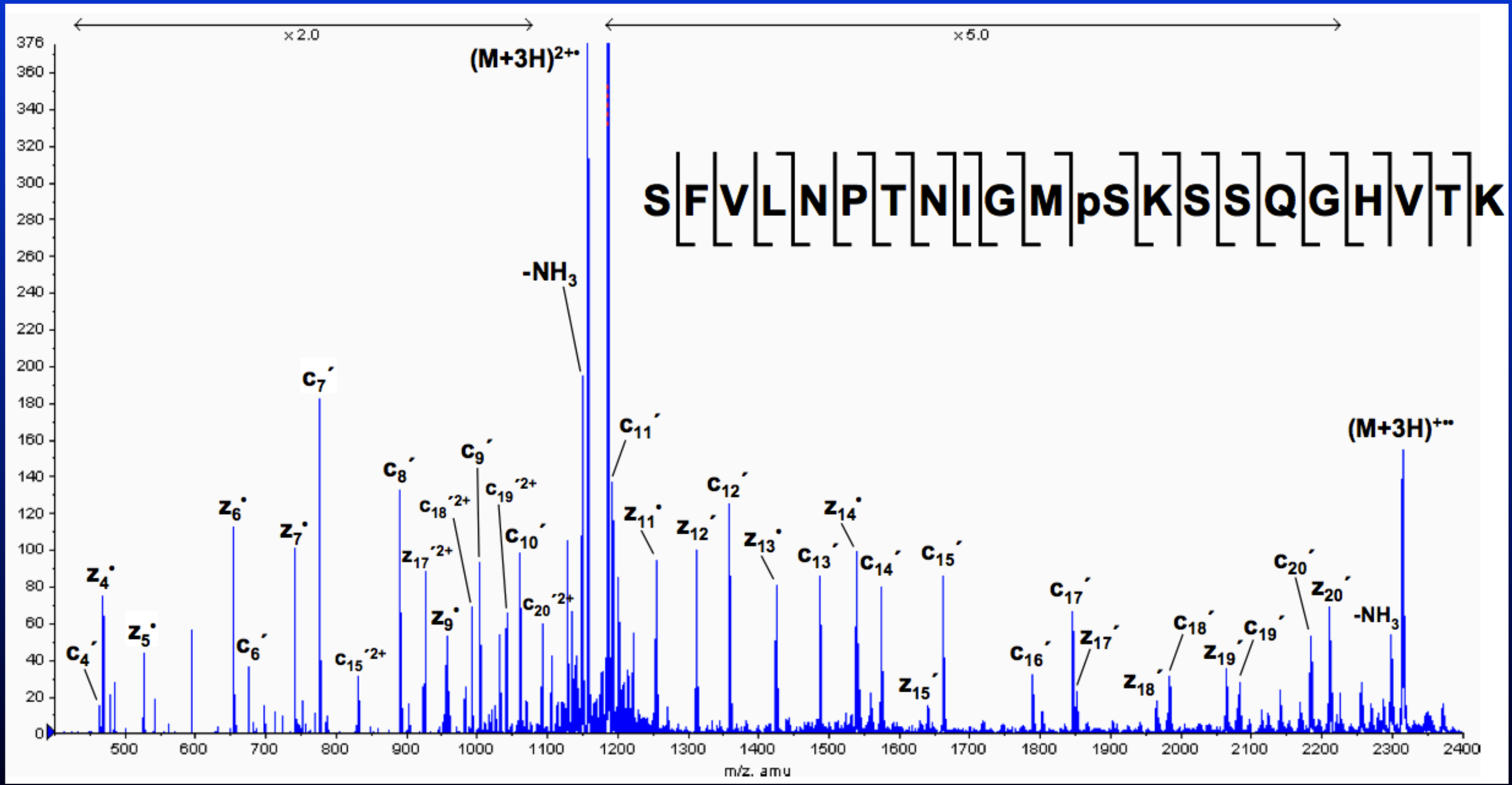
Results - Reproducibility



Atmospheric Pressure - Electron Capture Dissociation
Results - Glycosylation



Atmospheric Pressure - Electron Capture Dissociation
Results - Phosphopeptides



Coming up.....

Validate method further

Integrate with software

ECD-CID, ECD/ECD

Real Mixtures

HD exchange (Waters or Koenermann Alzeimer's)

Andrew Ross (phosphohistidines in bacterial proteins, methyglyoxal, ubiquitin)

HW improvements (U. Wuppertal and/or Ross)

Colby Methylation

J & J Pharma (glyco and phosopho)