Waters Select Series Cyclic IMS Qtof HDX-MS Workshop

24/10/24

Glenn Masson

SELECT SERIES

Waters Advanced Mass Spectrometry

Select Series Cyclic IMS w/ ECD and PAL 2000 Sample Handling Robot

- Overview of the mass spectrometer architecture
- Sample Handler layout, limitations, tricks and tips
- cIMS – how we use it
- ECD & ECD data analysis with Newmarket.

Apologies to Waters, and Malcolm Anderson specificity for stealing most of their slides!

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Select Series Cyclic IMS Instrument Overview

- Pepsin Pump (Auxiliary Solvent Manager (ASM)

- C18 Column Pump

(Binary Solvent Manager (BSM)
(A: 0.1% F.A B: ACN, 0.1% F.A)

Select Series Cyclic IMS Instrument Overview

- Sample Handling Robot
- Syringe Arm

- Room temp chamber
- Zero Degrees Chamber

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Select Series Cyclic IMS Instrument Overview

- HDX-Manager – temperature controlled pepsin and reverse-phase column chambers

Select Series Cyclic IMS Instrument Overview

- ESI Source
- Lock-mass (internal calibrant)

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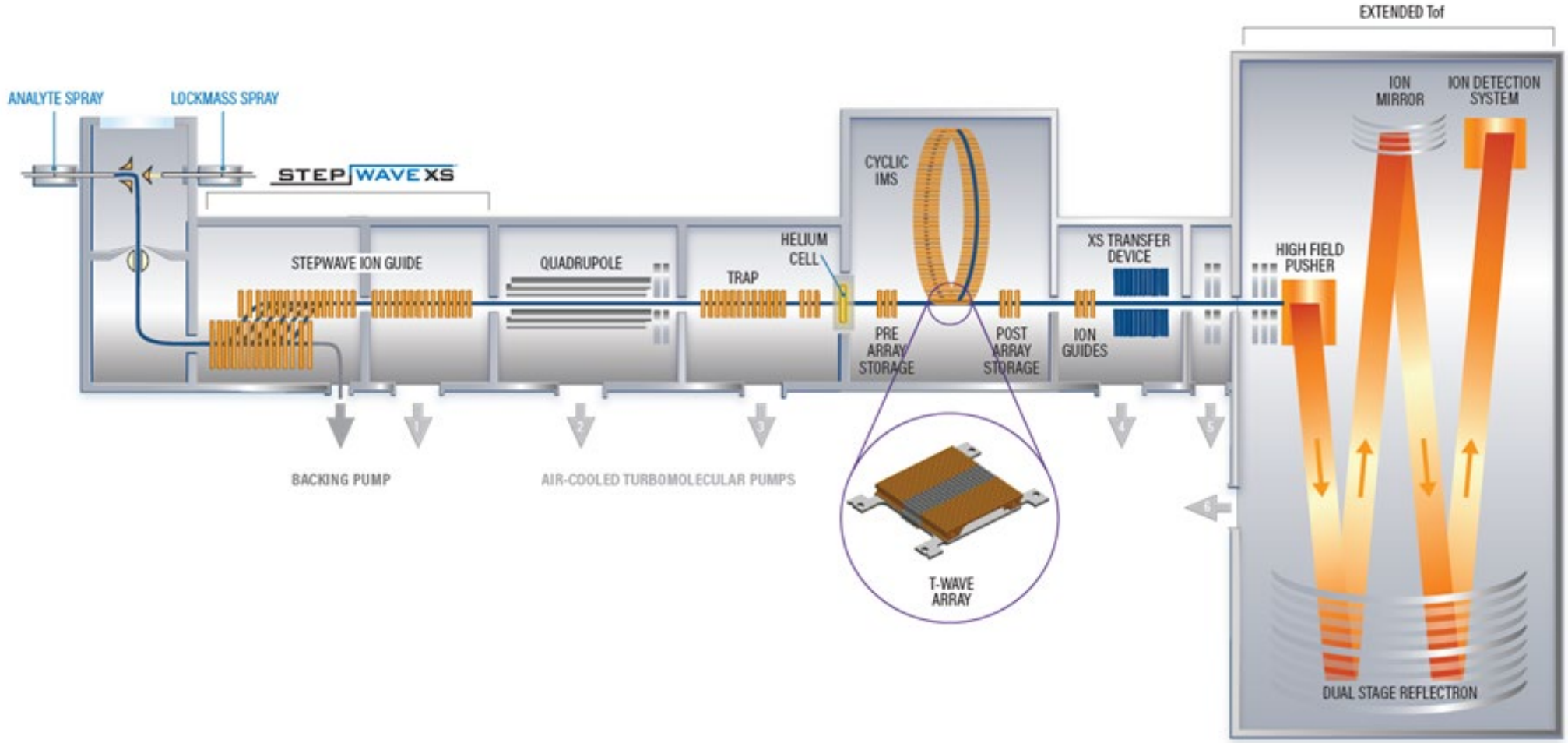

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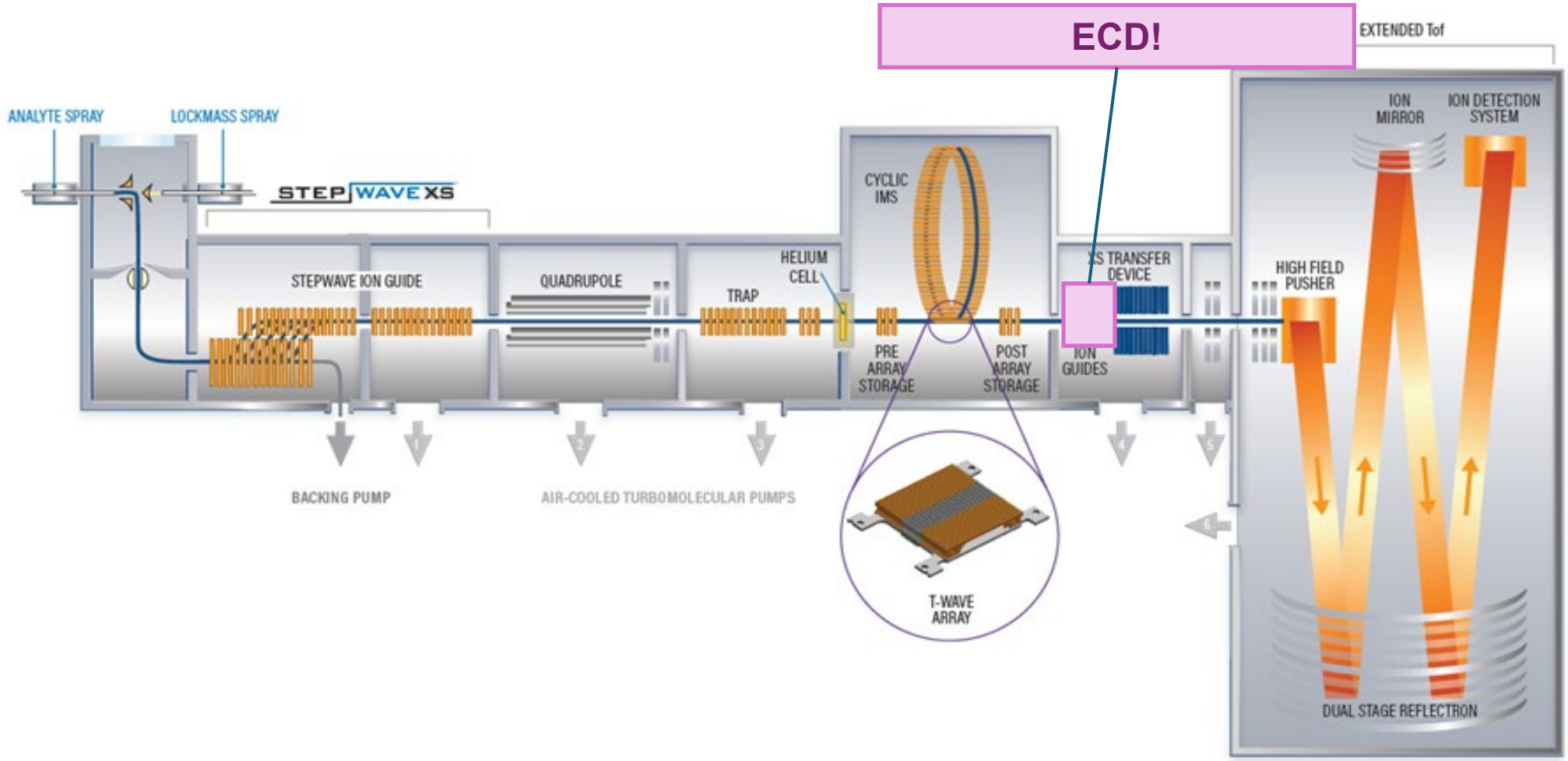

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Trajan Systems now come with their own HDX Manager box

However, I believe most of the software is largely maintained and the same…

This arm moves on this rail

Syringe

Room Temperature Box (RTB) is where is HDX takes place. Holds your D2O.

Zero Degree Box (ZDB) holds your protein and quench.

Typically, the robot

1) Puts quench in ZDB
2) Moves protein from ZDB to RTB
3) Waits a bit to equilibrate
4) Washes the syringe with pure D_2O
5) Adds D_2O buffer to RTB vial with protein
6) Mixes
7) Moves D_2O/protein mixture to ZTB quench
8) Mixes
9) Injects to HDX-Manager

Typically have two syringes: 10 µL and 250 µL

10 µL syringe moves your protein from the cold box to the room temperature box.

250 µL syringe dispenses the quench, mixes the D_2O and injects the sample.

Syringe Parking

Syringe

Wash Station

Syringe

Considerations (Our Experience):

- Fastest Exchange reaction it can do is ~30s, but there's quite a bit of error there. In reality, it's 1 min.
- Once you factor in dead volumes etc., you're looking at using at *least twice as much protein.*
- Some quenches (6M GdnCl) really don't like sitting at 0 degrees for hours on end and precipitate and clog the system….
- It uses two glass vials for each injection. For some reason, we get much less carry over between samples if we run a "proper" blank (i.e. all the injections etc., just no protein) between samples…~4 vials per sample…

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SKU: 186000384C

LCGC Certified Clear Glass 12 x 32 mm Screw Neck Vial, Total Recovery, with Cap and PTFE/Silicone Septum, 1 mL Volume, 100/pk

Your Price
152.00 GBP

In Stock
Order Now

1

ADD TO CART

LCGC Certified Clear Glass Vial | 12 x 32mm | 186000384C

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- Mixing can be problematic.

- You can customise quite a bit with vial locations, feels a bit scary though!
- Running the instrument overnight with 40+ samples is great in terms of efficiency

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- Running the instrument overnight with 40+ samples is great in terms of efficiency

- Practical Tips: Run a single sample at 3 pm, check it's all working.
- *Then* set up the complete run (avoids dispensing 40 quench vials)

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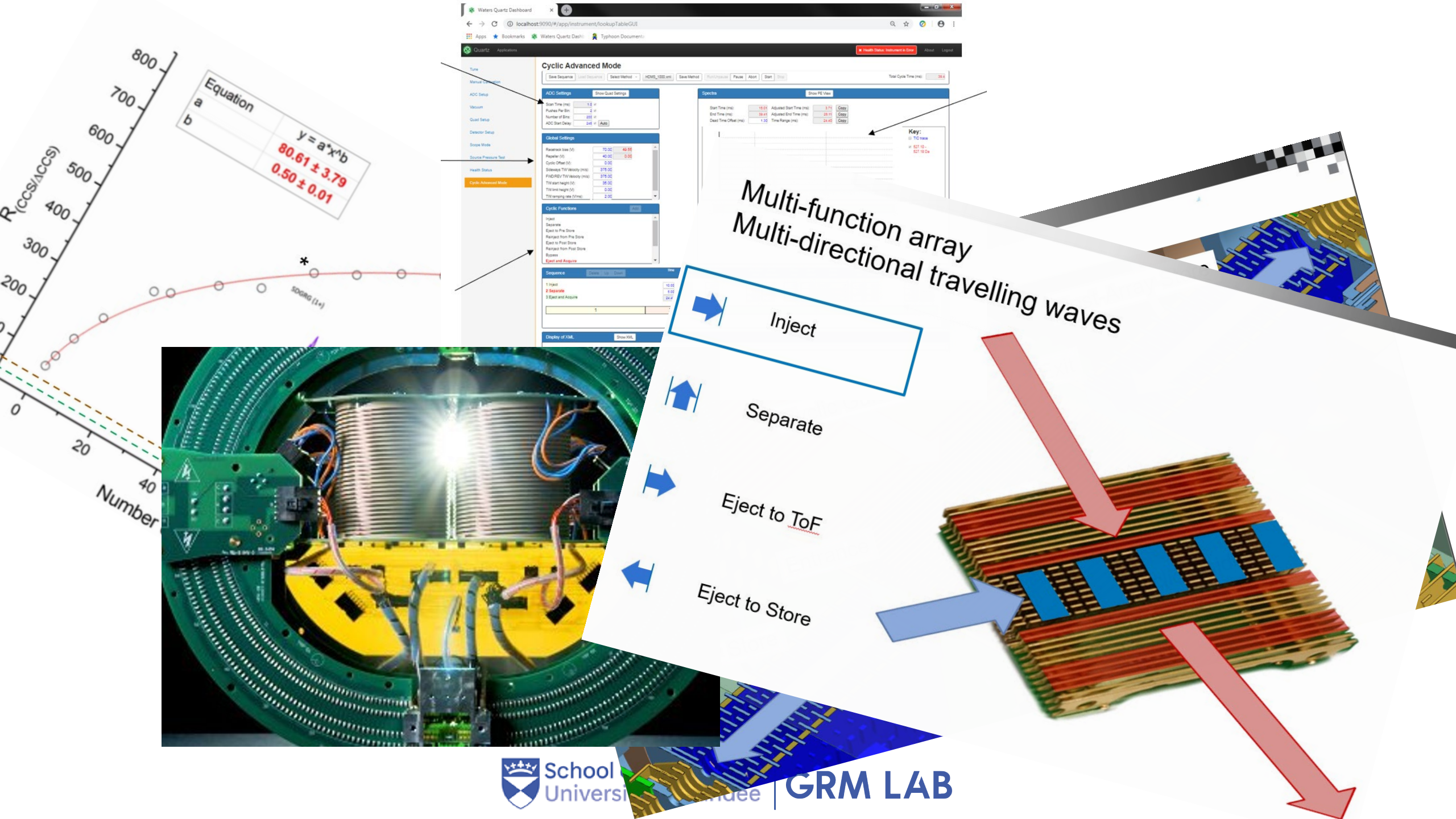
Equation
a
b
y = a*x^b
80.61 ± 3.79
0.50 ± 0.01
R(CCS/ΔCCS)
Number
Waters Quartz Dashboard
localhost:9090/#/app/instrument/lookupTableGUI
Cyclic Advanced Mode
Multi-function array
Multi-directional travelling waves
Inject
Separate
Eject to ToF
Eject to Store
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We just use it as a linear IMS

very good

i.e. all the peptides go around once

Why don’t we do multiple passes?

- Most of our analytes (i.e. peptides) have high redundancy, and three distinguishing features
  - Retention Time
  - m/z
  - Ion Mobility
- We’ve yet to have a situation where a “mission critical” peptide is problematic because of a lack of Ion Mobility resolution.
- Doesn’t mean this won’t happen in the future.

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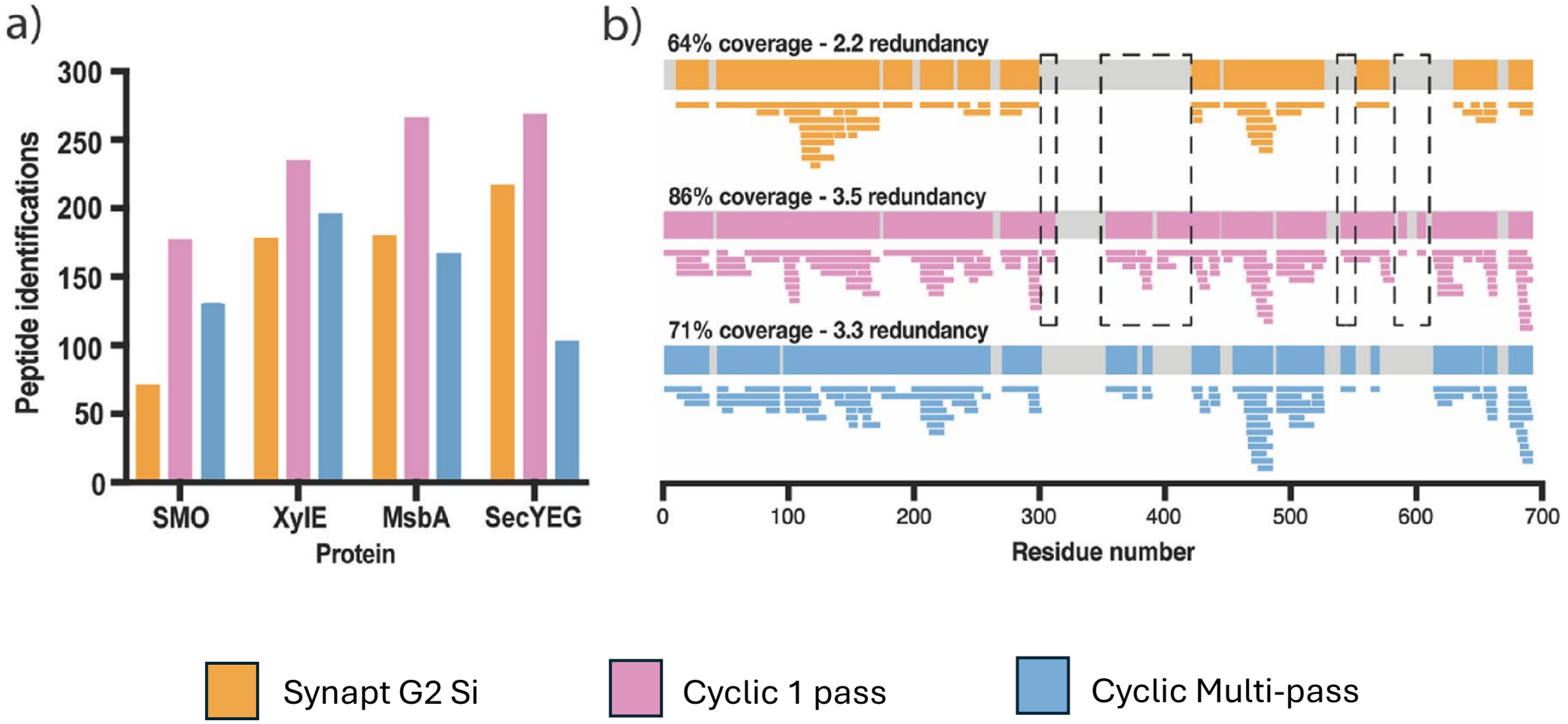

Griffiths, Damon et al. “Cyclic Ion Mobility for Hydrogen/Deuterium Exchange-Mass Spectrometry Applications.” *Analytical chemistry* vol. 96,15 (2024): 5869-5877. doi:10.1021/acs.analchem.3c05753

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ECD on the cIMS

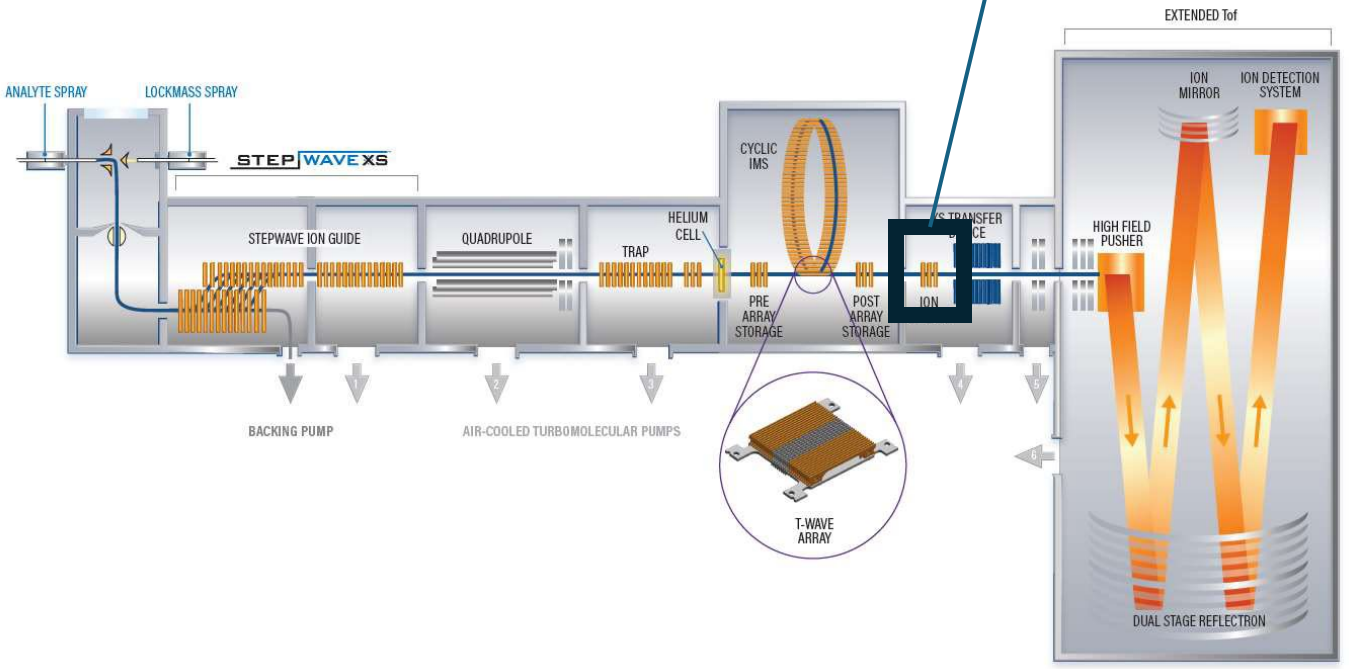
Electron Capture Dissociation for the “soft” fragmentation of peptides without deuterium signal randomisation (scrambling).

Ours located *after* the cIMS

Optimisation of lens and filament current done using P1 peptide to check scrambing

eMSion

ECD for the Masses



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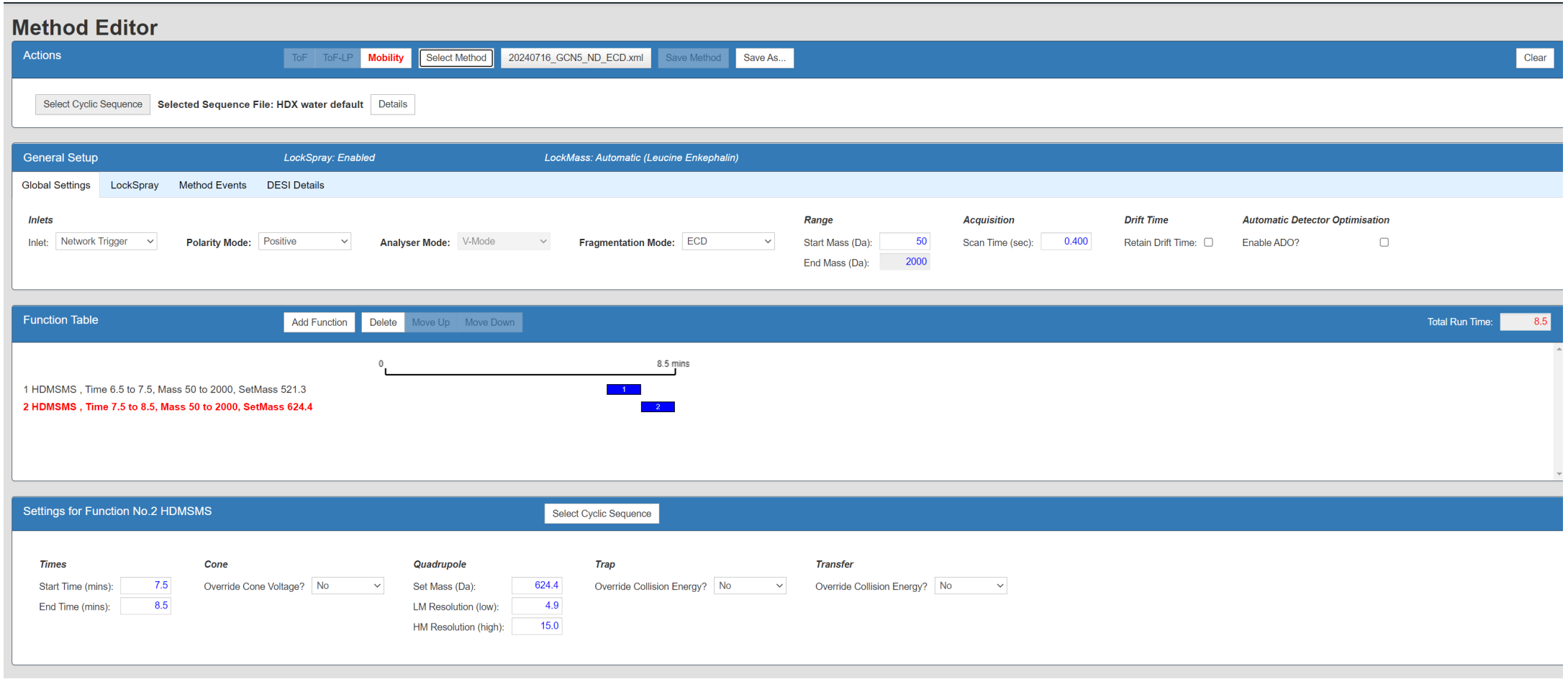
ECD on the cIMS

We do *targeted* ECD on certain precursor peptides

- Want high abundance +3/+4 peptides



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Data Courtesy of De Lin (Drug Discovery Unit)

ECD on the cIMS

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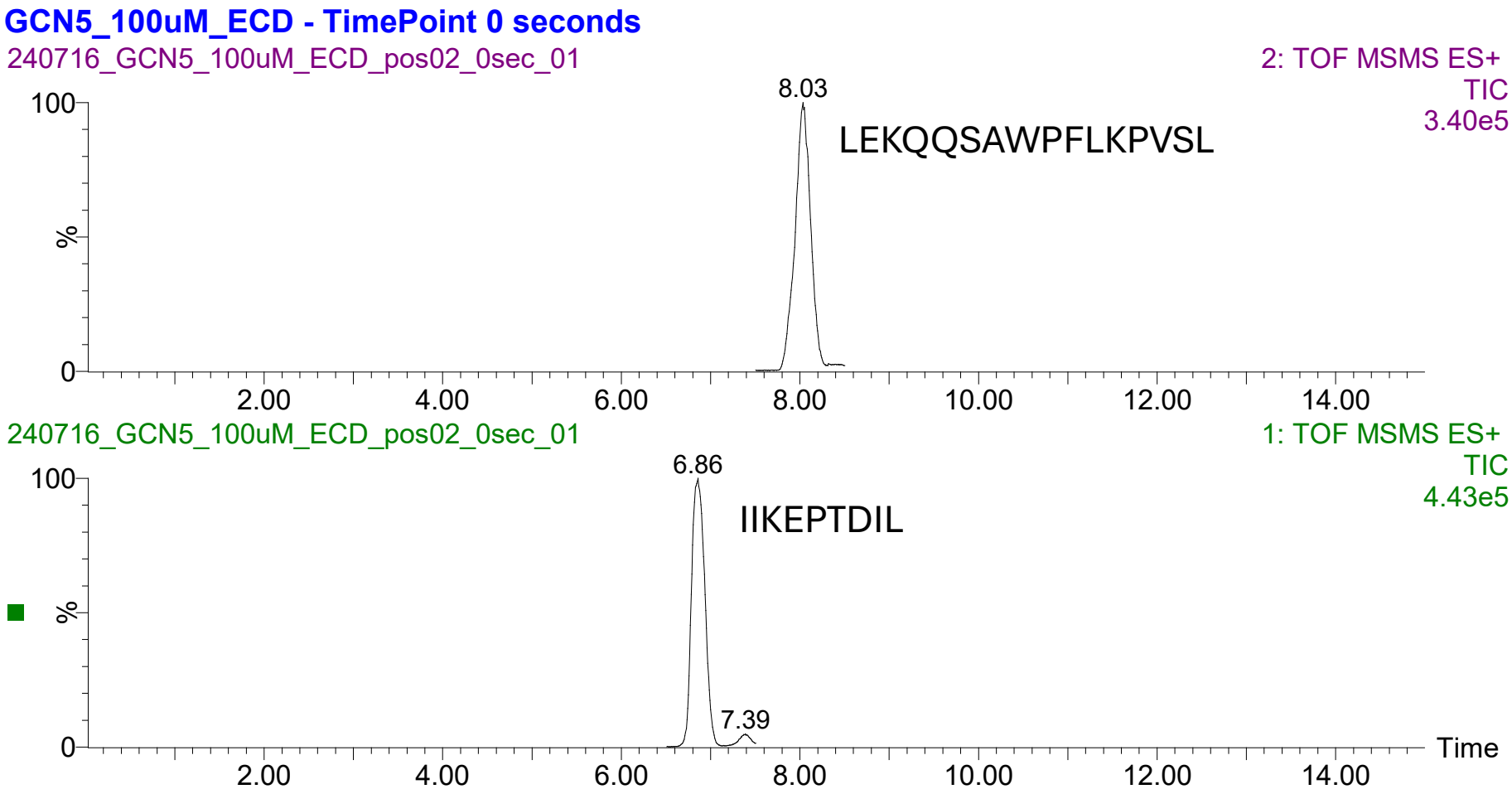

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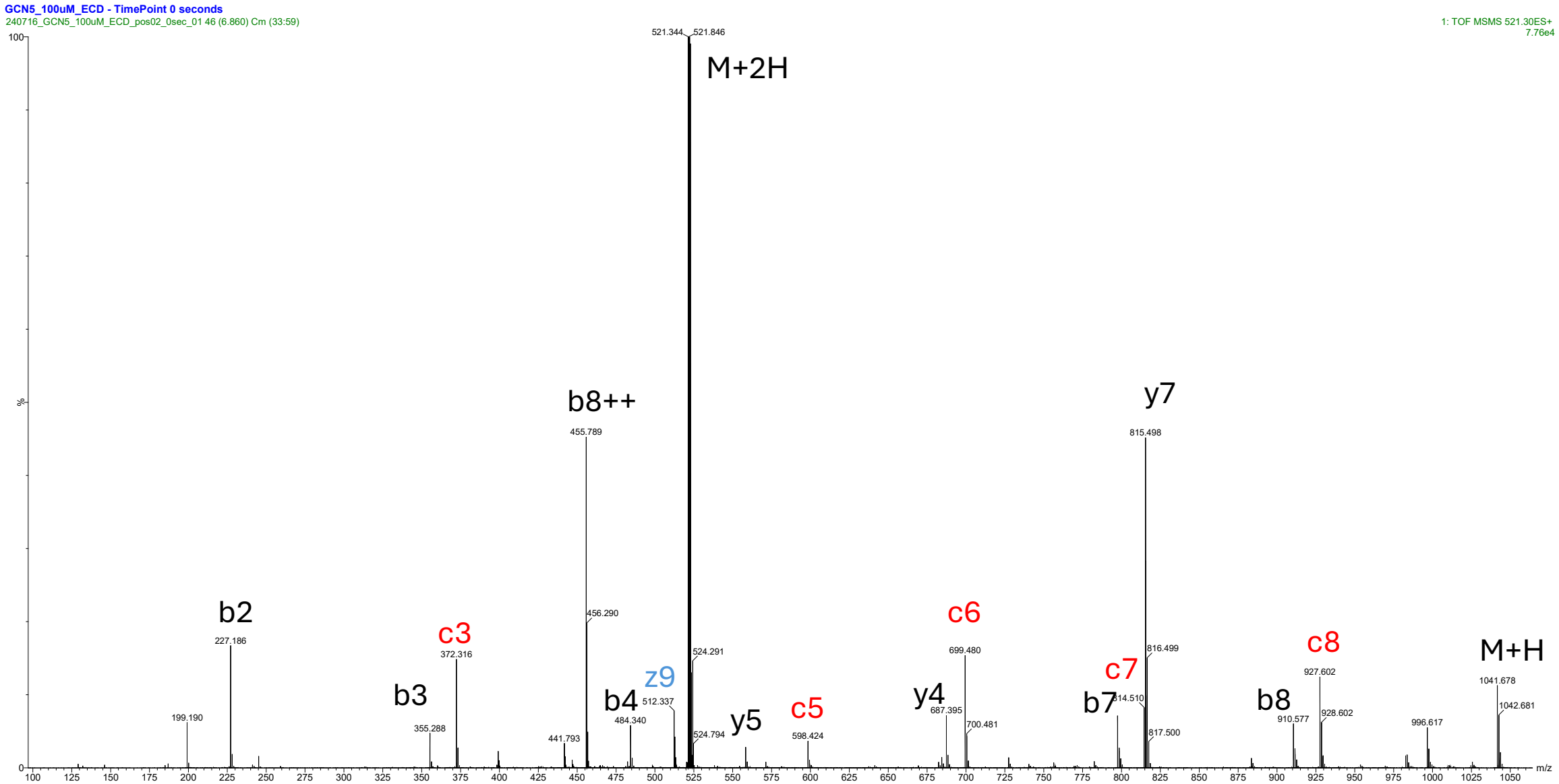
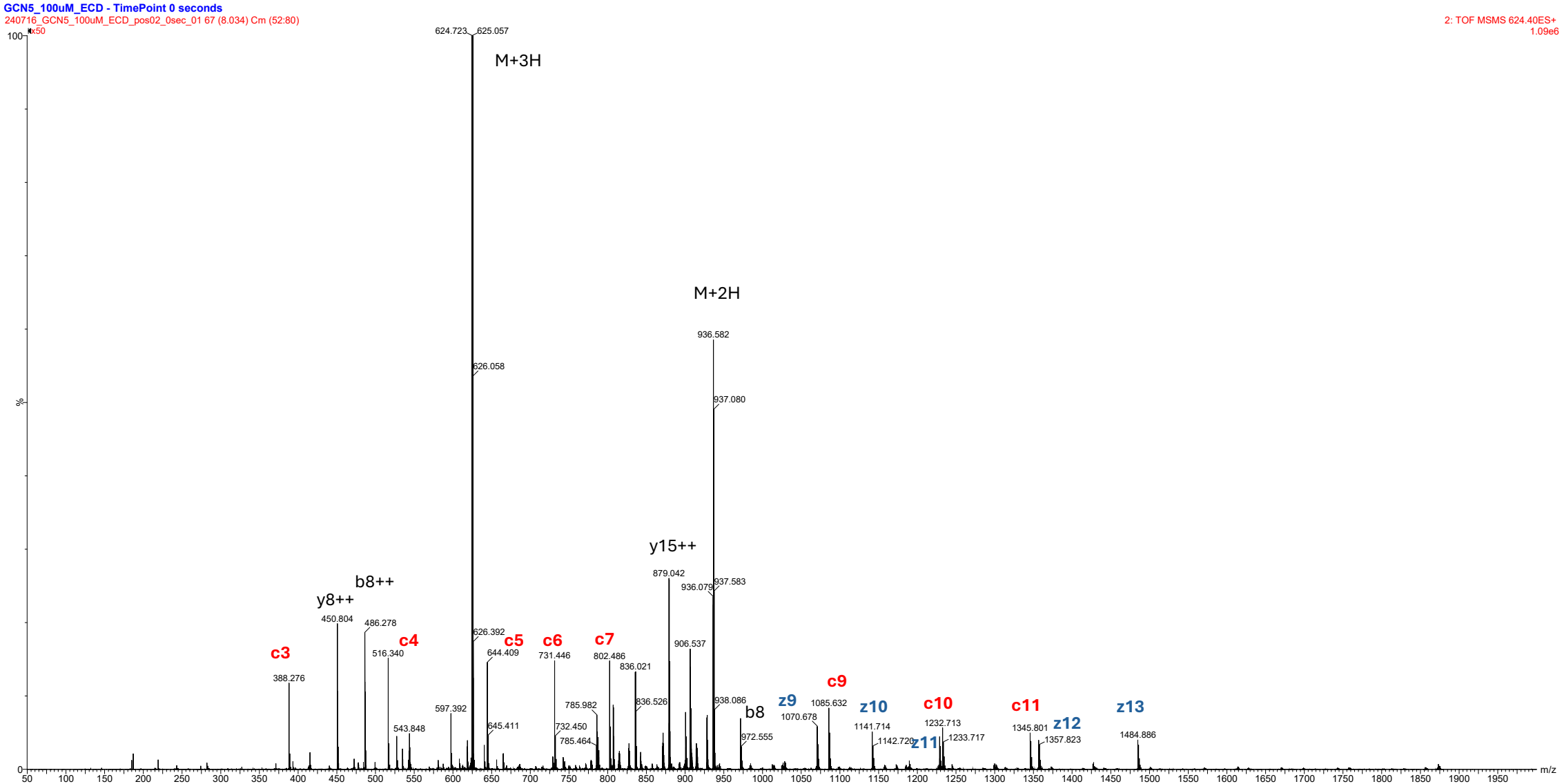
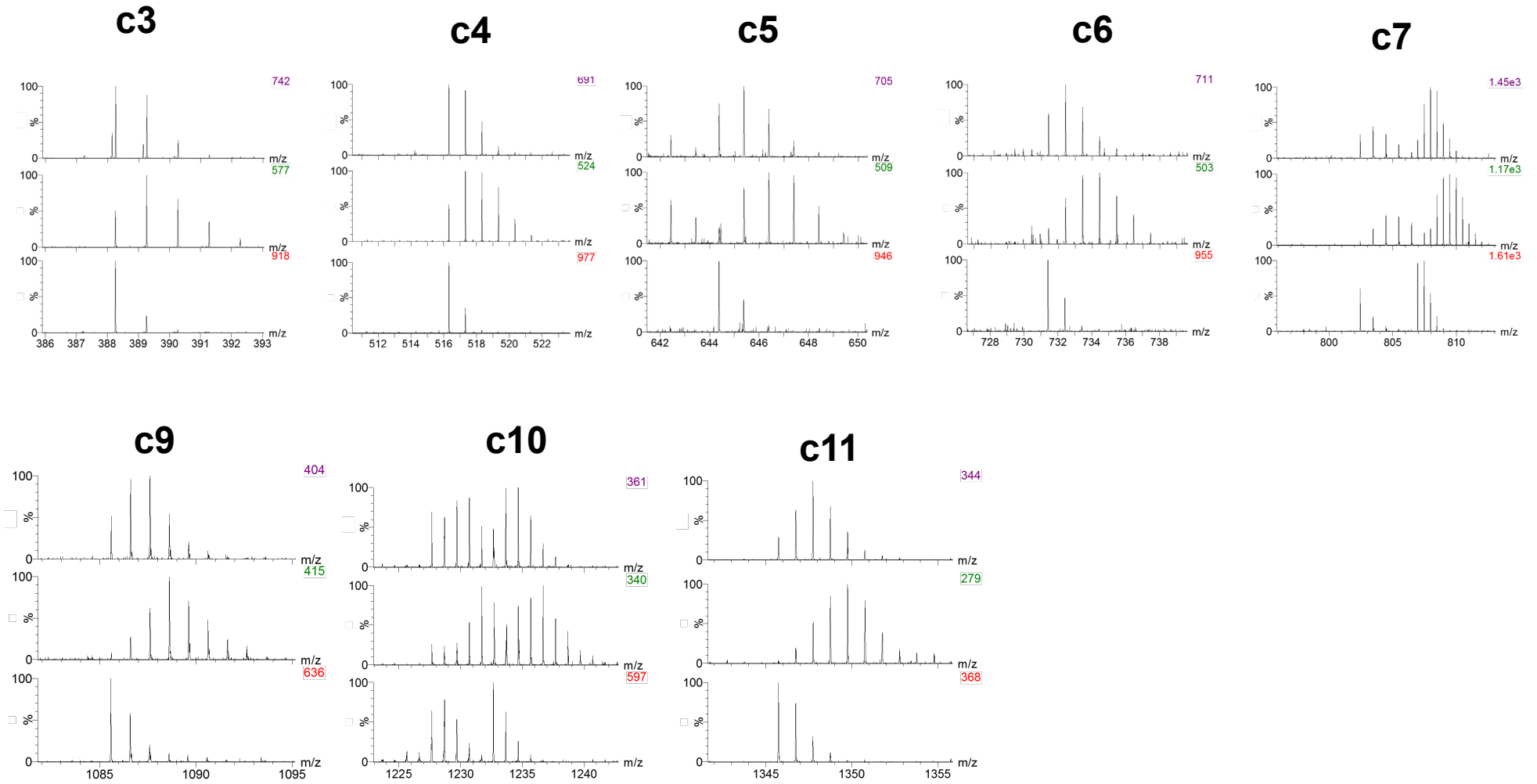
MSMS – peptide IIKEPTDIL



MSMS – peptide LEKQQSAWPFLKPVSL



MSMS – peptide llKEPTDIL c ion series



Newmarket – a new HDX-MS Data Analysis Software from Waters with ECD capability