

Waters Select Series Cyclic IMS Qtof

HDX-MS Workshop

24/10/24

Glenn Masson



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!

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



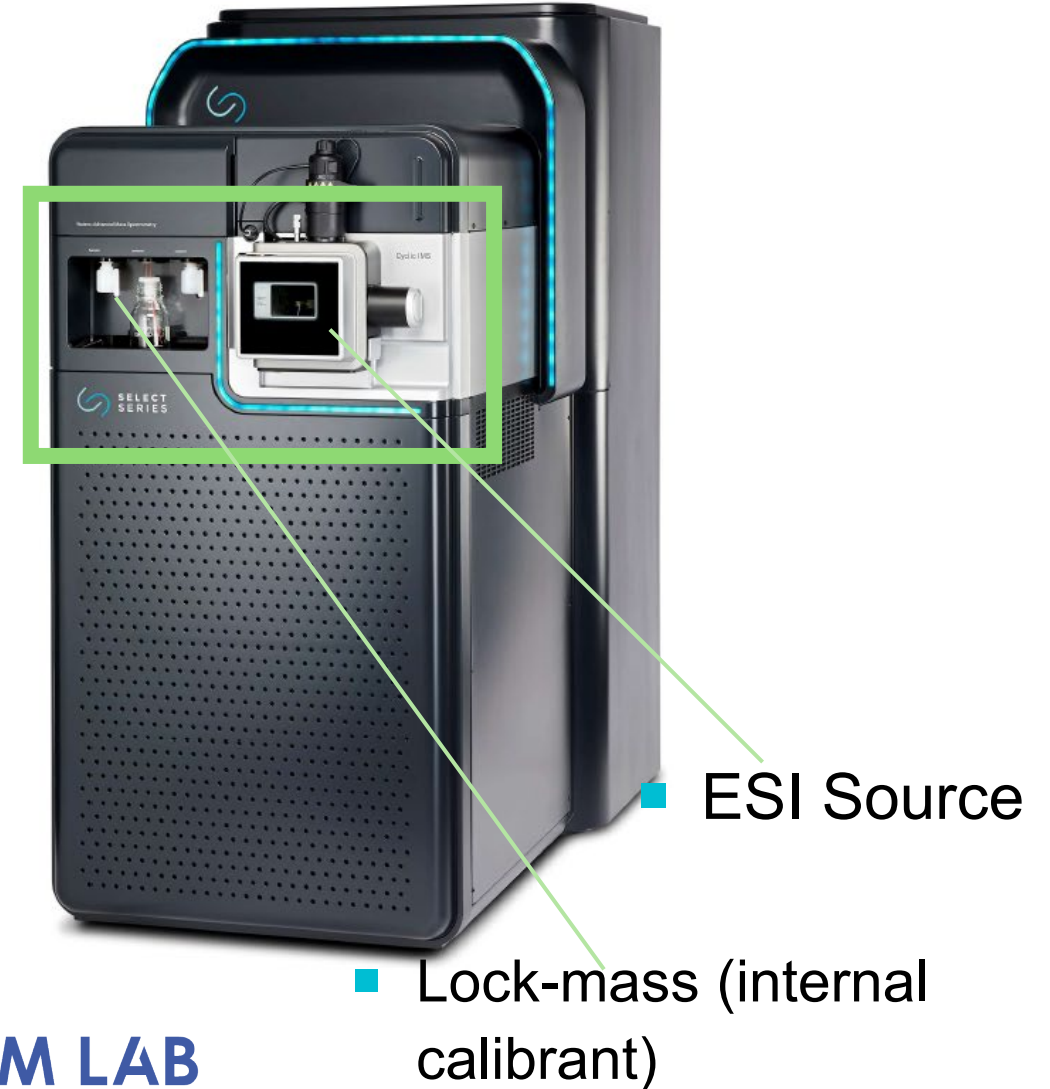
Select Series Cyclic IMS Instrument Overview



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



Select Series Cyclic IMS Instrument Overview



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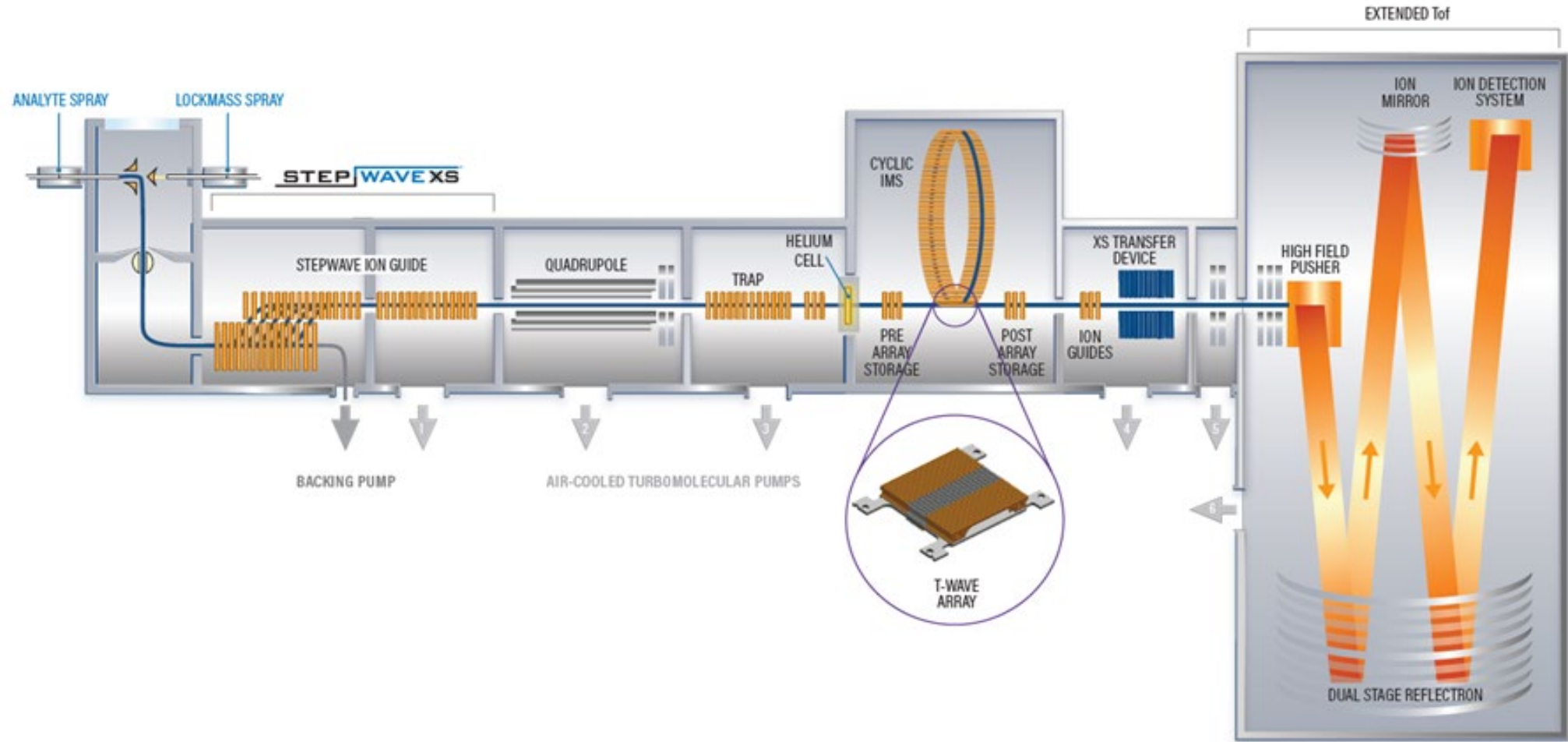
■ TOF



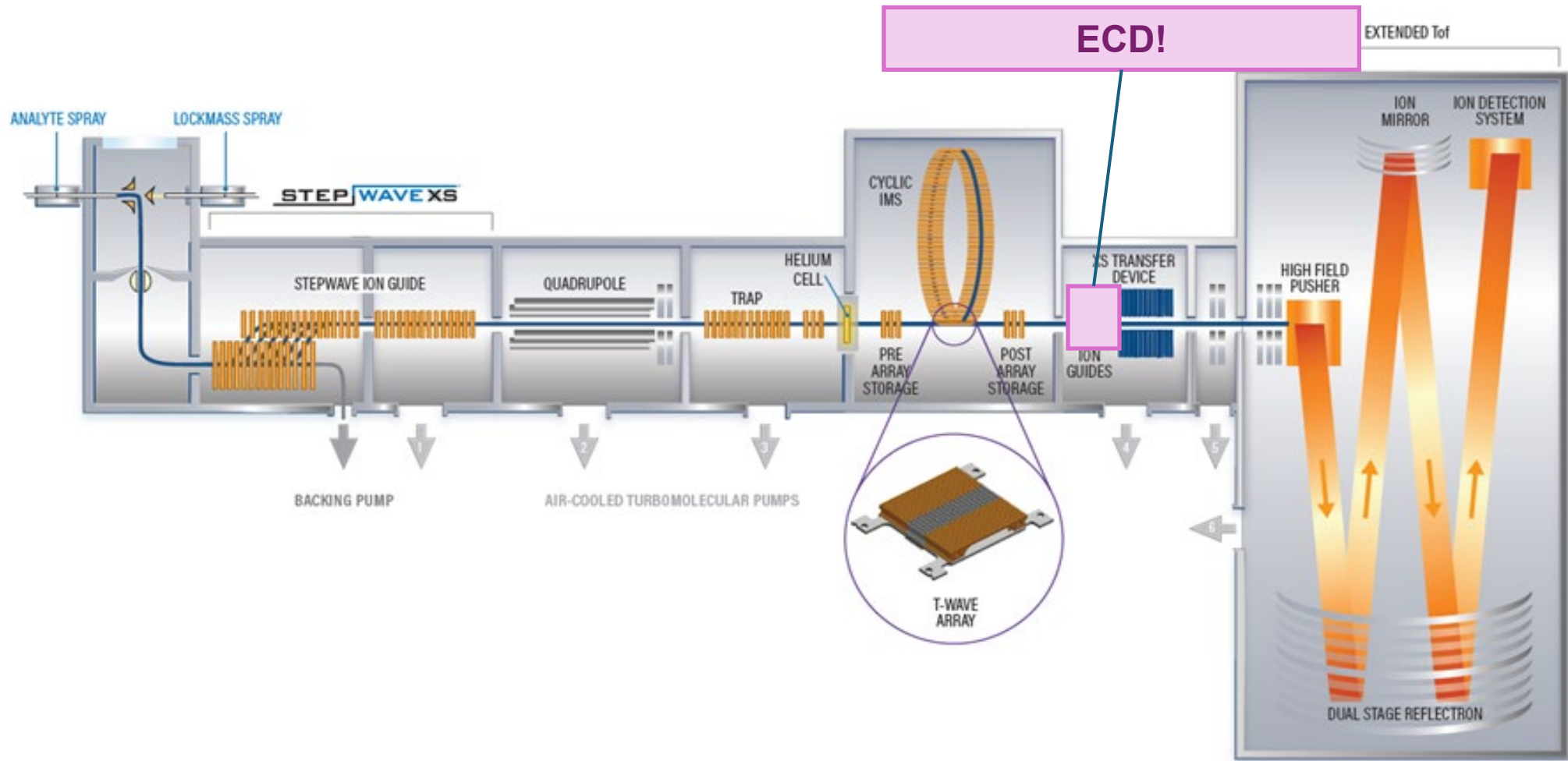
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N.B. I am fairly certain that our set up with a Waters HDX-Manager system with a Trajan LEAP PAL HDX (“Compact”) system although only 3 years old, is no longer available



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Trajan Systems now come with their own HDX Manager box



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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 D₂O
- 5) Adds D₂O buffer to RTB vial with protein
- 6) Mixes
- 7) Moves D₂O/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 ✓
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ADD TO CART



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



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- Injection port of the HDX Manager gets much more wear and tear from the robot.
- 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



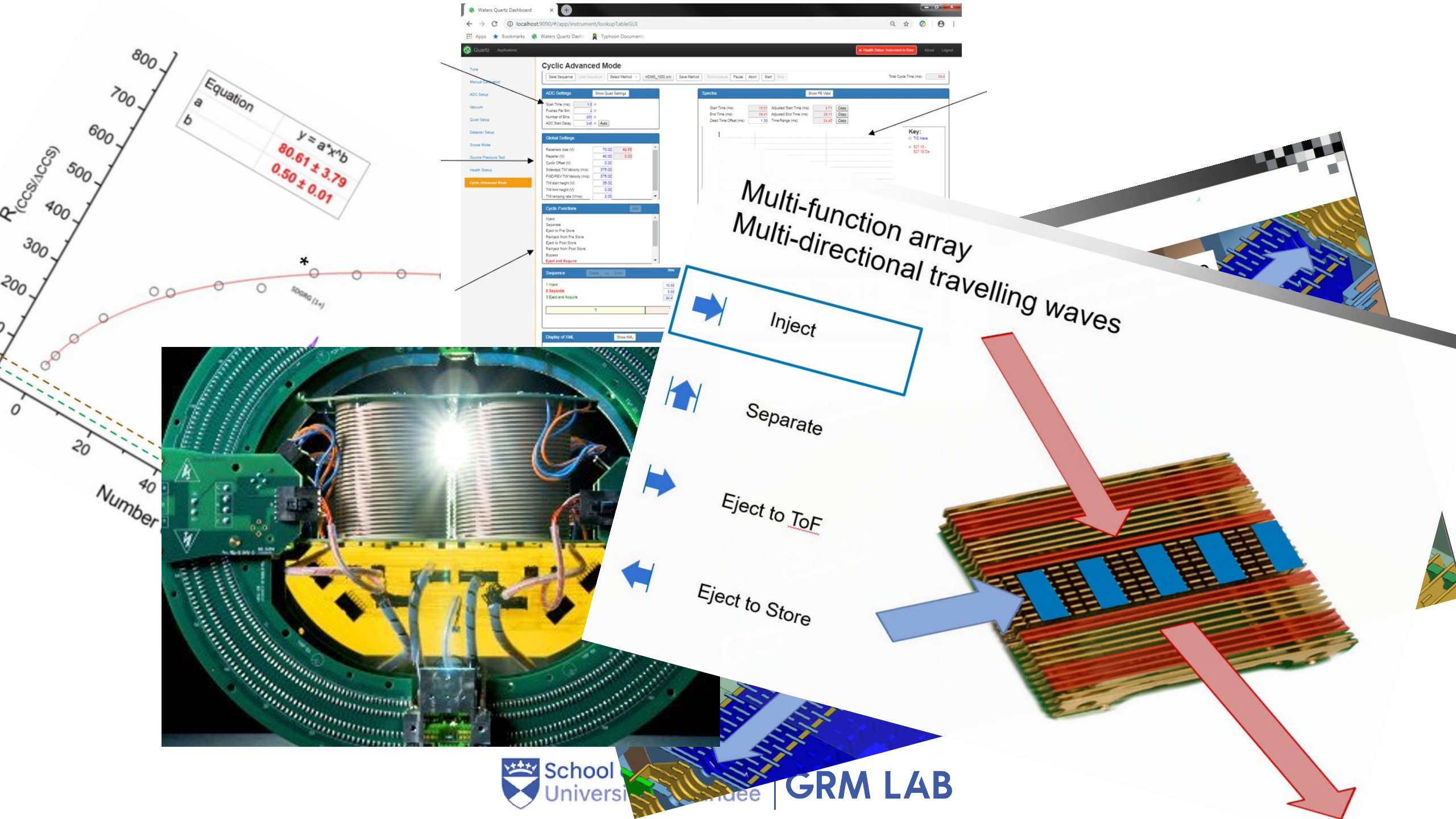
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- You can customise quite a bit with vial locations, feels a bit scary though!
- Running the instrument overnight with 40+ samples is great in terms of efficiency
- Practical Tips: Run a single sample at 3 pm, check it's all working.
- *Then* set up the complete run (avoids dispensing 40 quench vials)



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Equation

$$y = a \cdot x^b$$

80.61 ± 3.79
0.50 ± 0.01

Waters Quartz Dashboard

localhost:9090/#/app/instrument/lookupTableGUI

Quartz Applications

Cyclic Advanced Mode

Save Sequence | Load Sequence | Select Method | HPLC_1000.um | Save Method | Run/Shutdown | Pause | Abort | Start | Stop | Total Cycle Time (min): 38.4

ADC Settings | Show Grad Settings

Start Time (min): 1.0 | Adjusted Start Time (min): 1.11 | Copy

End Time (min): 38.41 | Adjusted End Time (min): 28.11 | Copy

Number of Rows: 200 | Time Range (min): 24.05 | Copy

Dead Time Offset (min): 1.00

Global Settings

Flowback Rate (l/min): 10.00 | 40.00

Paperlet (l/min): 40.00 | 2.00

Cyclic Offset (min): 2.00

Sideflow TIV Velocity (mm/s): 375.00

Flowback TIV Velocity (mm/s): 375.00

TIV start height (mm): 38.00

TIV end height (mm): 2.00

TIV ramping rate (mm/s): 2.00

Cyclic Functions

Inject

Separate

Eject to Pre Store

Rampback from Pre Store

Eject to Post Store

Rampback from Post Store

Eject

Eject and Acquire

Sequences

1 Inject | 10.00

2 Separate | 2.00

3 Eject and Acquire | 24.41

Display of JML | Show JML

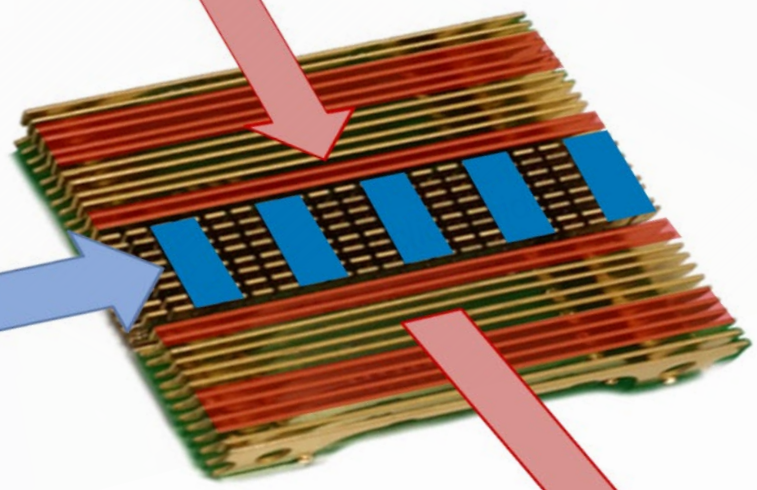
Multi-function array
Multi-directional travelling waves

Inject

Separate

Eject to ToF

Eject to Store





very good

We just use it as a linear IMS

i.e. all the peptides go around once



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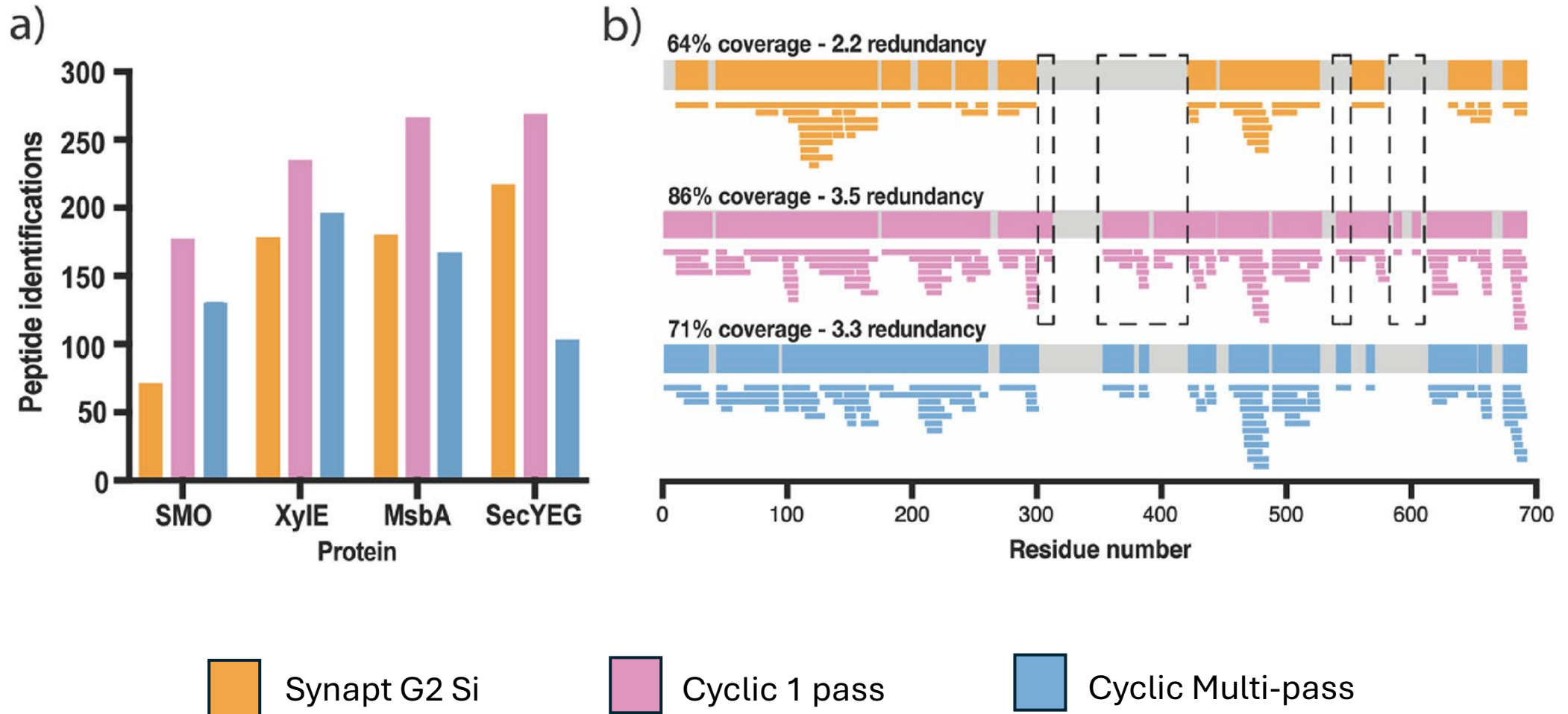
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Why don't we do multiple passes?

- 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.



Why don't we do multiple passes?



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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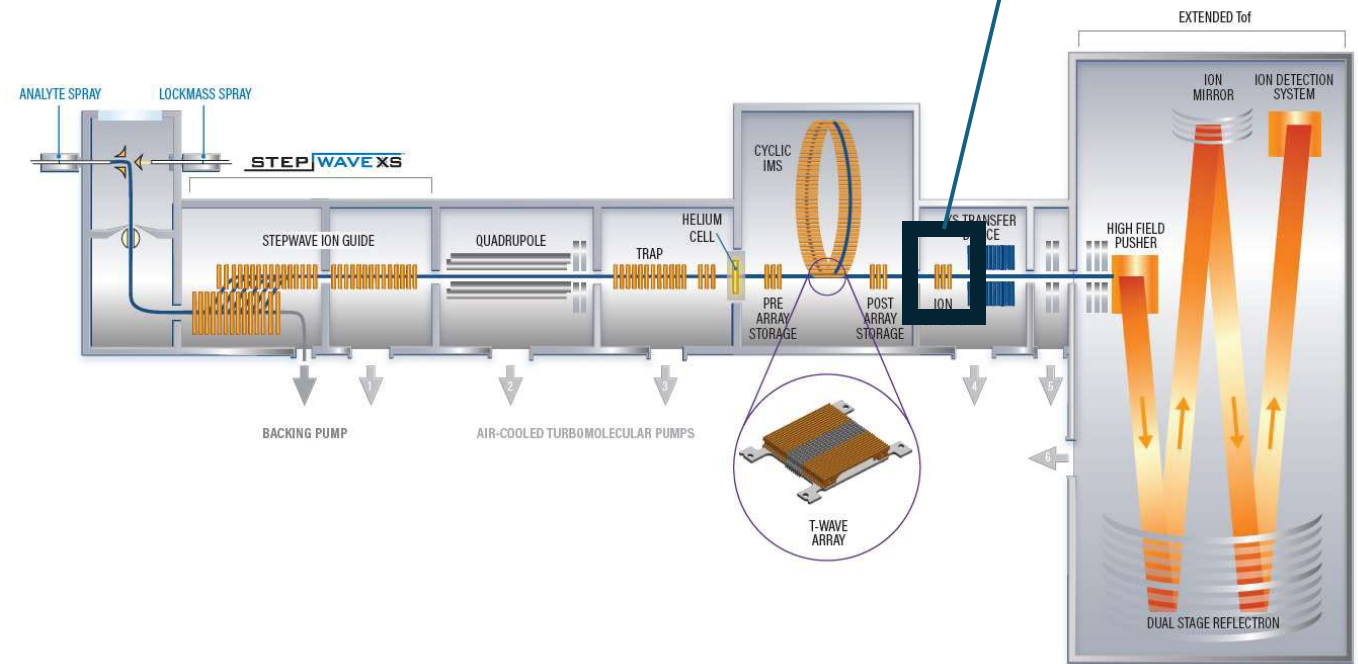
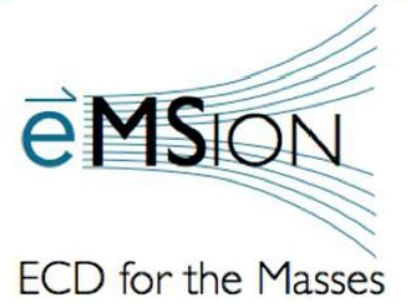
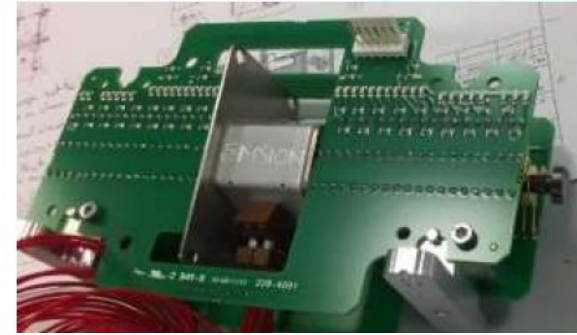
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ECD on the cIMS

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 scrambling



ECD on the cIMS

We do *targeted* ECD on certain precursor peptides

- Want high abundance +3/+4 peptides

Method Editor

Actions: ToF ToF-LP **Mobility** Select Method 20240716_GCN5_ND_ECD.xml Save Method Save As... Clear

Select Cyclic Sequence Selected Sequence File: HDX water default Details

General Setup LockSpray: Enabled LockMass: Automatic (Leucine Enkephalin)

Global Settings LockSpray Method Events DESI Details

Inlets Inlet: Network Trigger Polarity Mode: Positive Analyser Mode: V-Mode Fragmentation Mode: ECD

Range Start Mass (Da): 50 End Mass (Da): 2000

Acquisition Scan Time (sec): 0.400

Drift Time Retain Drift Time:

Automatic Detector Optimisation Enable ADO?

Function Table Add Function Delete Move Up Move Down Total Run Time: 8.5

0 8.5 mins

1 HDMSMS , Time 6.5 to 7.5, Mass 50 to 2000, SetMass 521.3

2 HDMSMS , Time 7.5 to 8.5, Mass 50 to 2000, SetMass 624.4

Settings for Function No.2 HDMSMS Select Cyclic Sequence

Times Start Time (mins): 7.5 End Time (mins): 8.5

Cone Override Cone Voltage? No

Quadrupole Set Mass (Da): 624.4 LM Resolution (low): 4.9 HM Resolution (high): 15.0

Trap Override Collision Energy? No

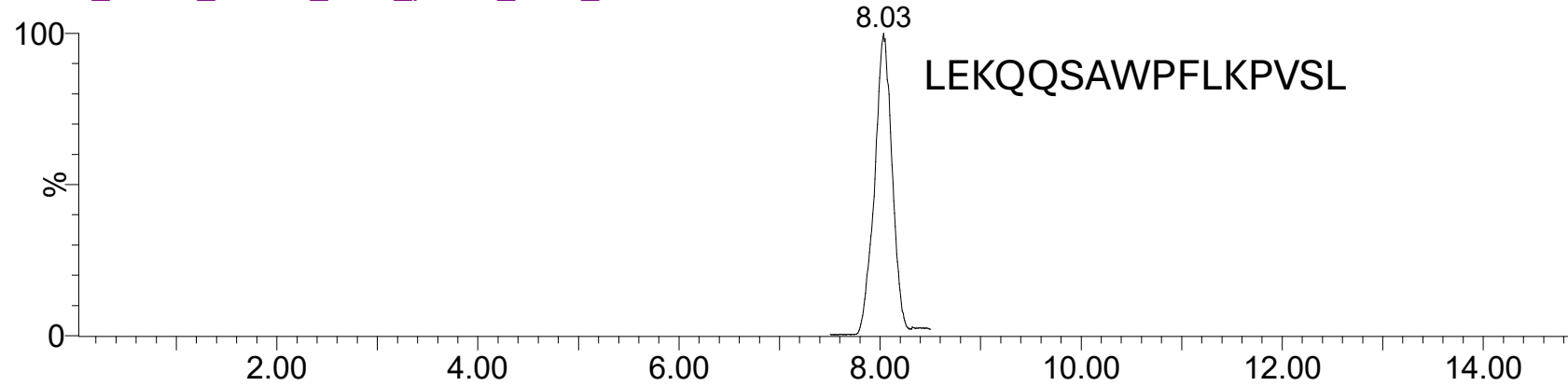
Transfer Override Collision Energy? No

ECD on the cIMS

We do *targeted* ECD on certain precursor peptides

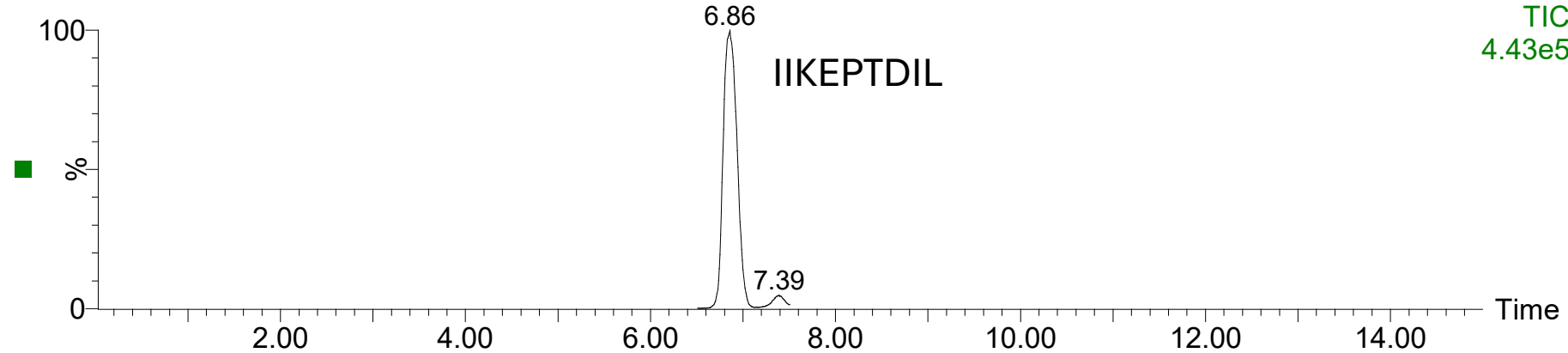
GCN5_100uM_ECD - TimePoint 0 seconds

240716_GCIN5_100uM_ECD_pos02_0sec_01



2: TOF MSMS ES+
TIC
3.40e5

240716_GCIN5_100uM_ECD_pos02_0sec_01

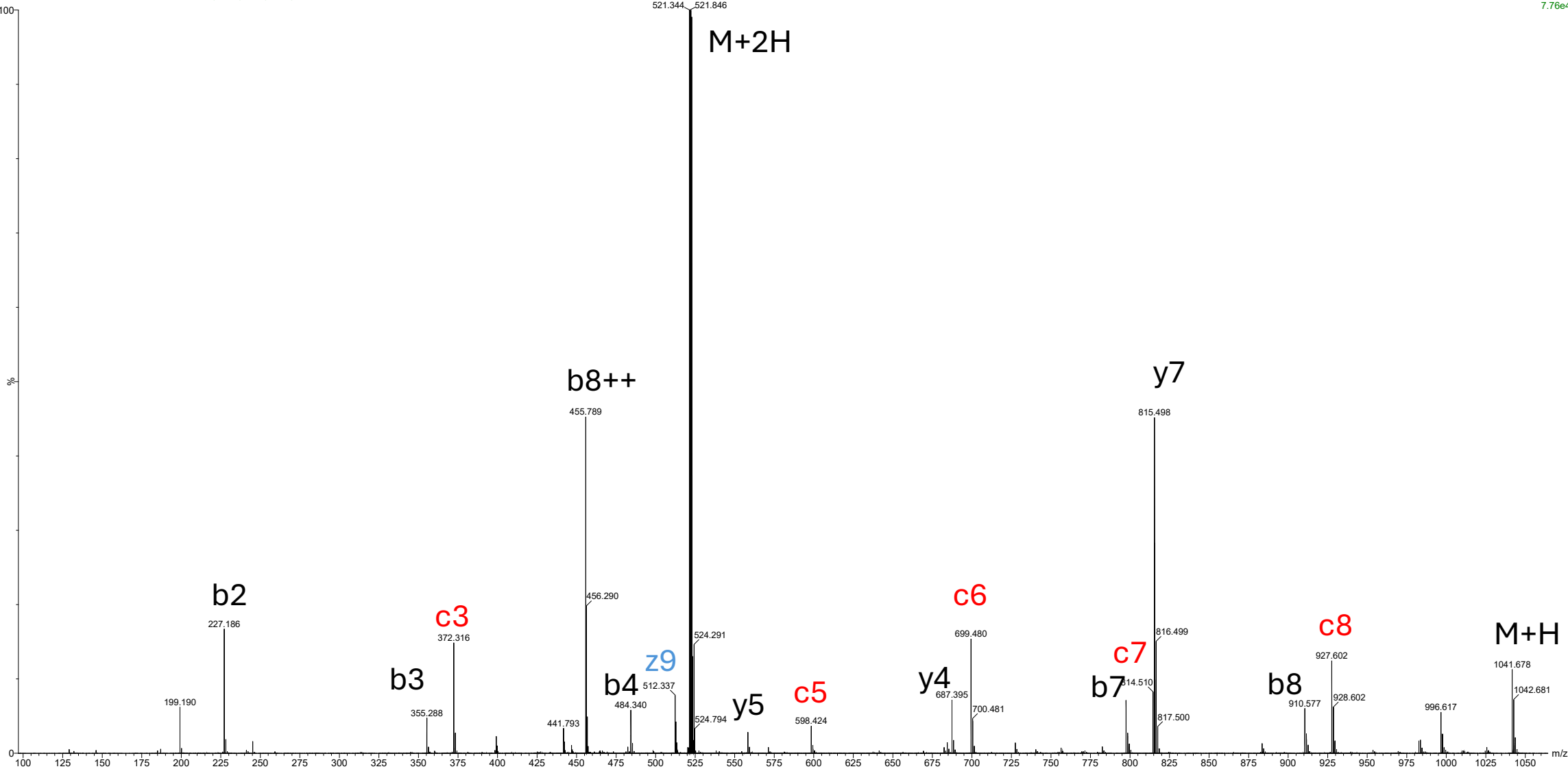


1: TOF MSMS ES+
TIC
4.43e5

MSMS – peptide IIKEPTDIL

GCN5_100uM_ECD - TimePoint 0 seconds
240716_GC5_100uM_ECD_pos02_0sec_01.46 (6.860) Cm (33:59)

1: TOF MSMS 521.30ES+
7.76e4

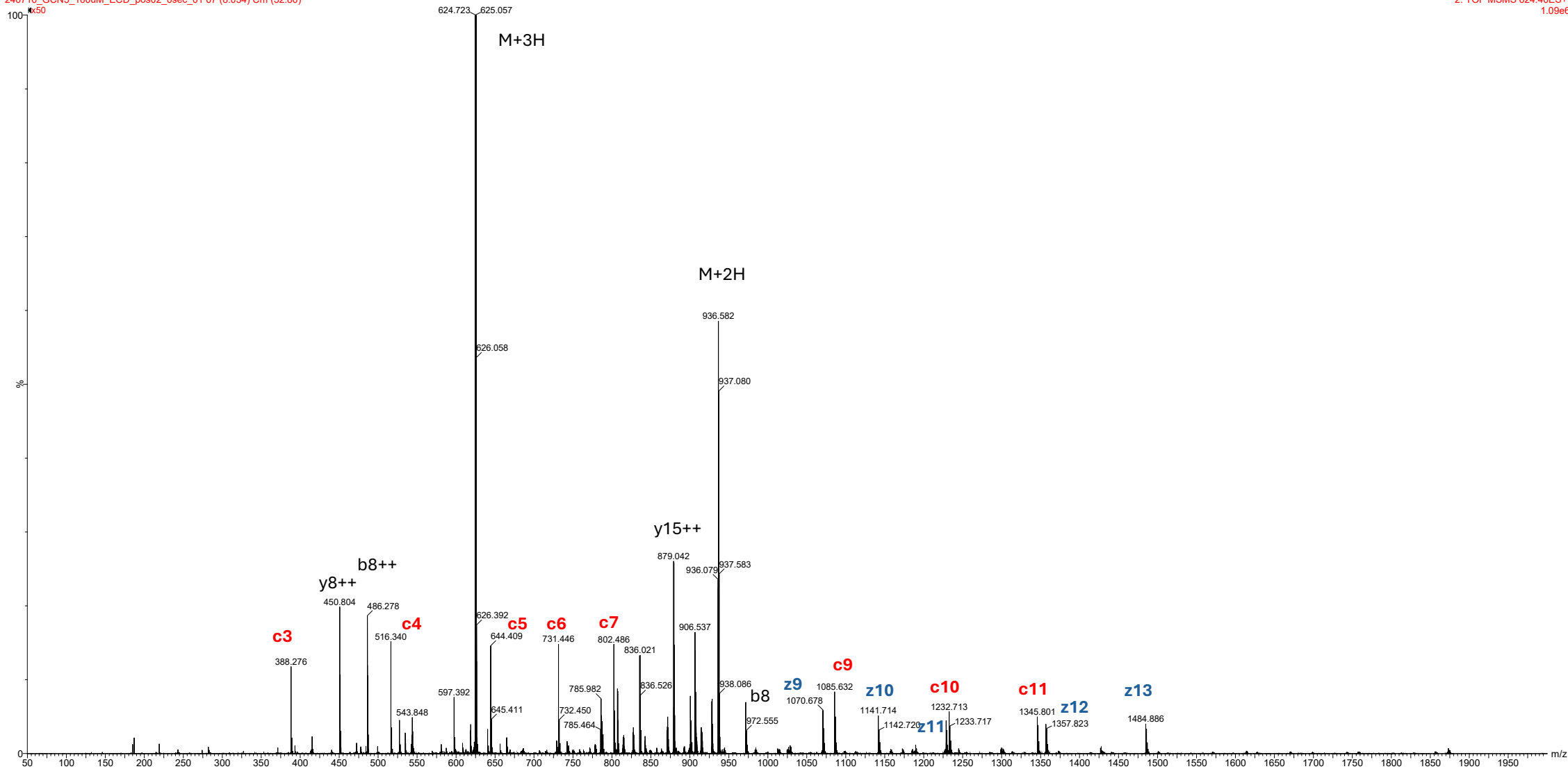


MSMS – peptide LEKQSAWPFLKPVSL

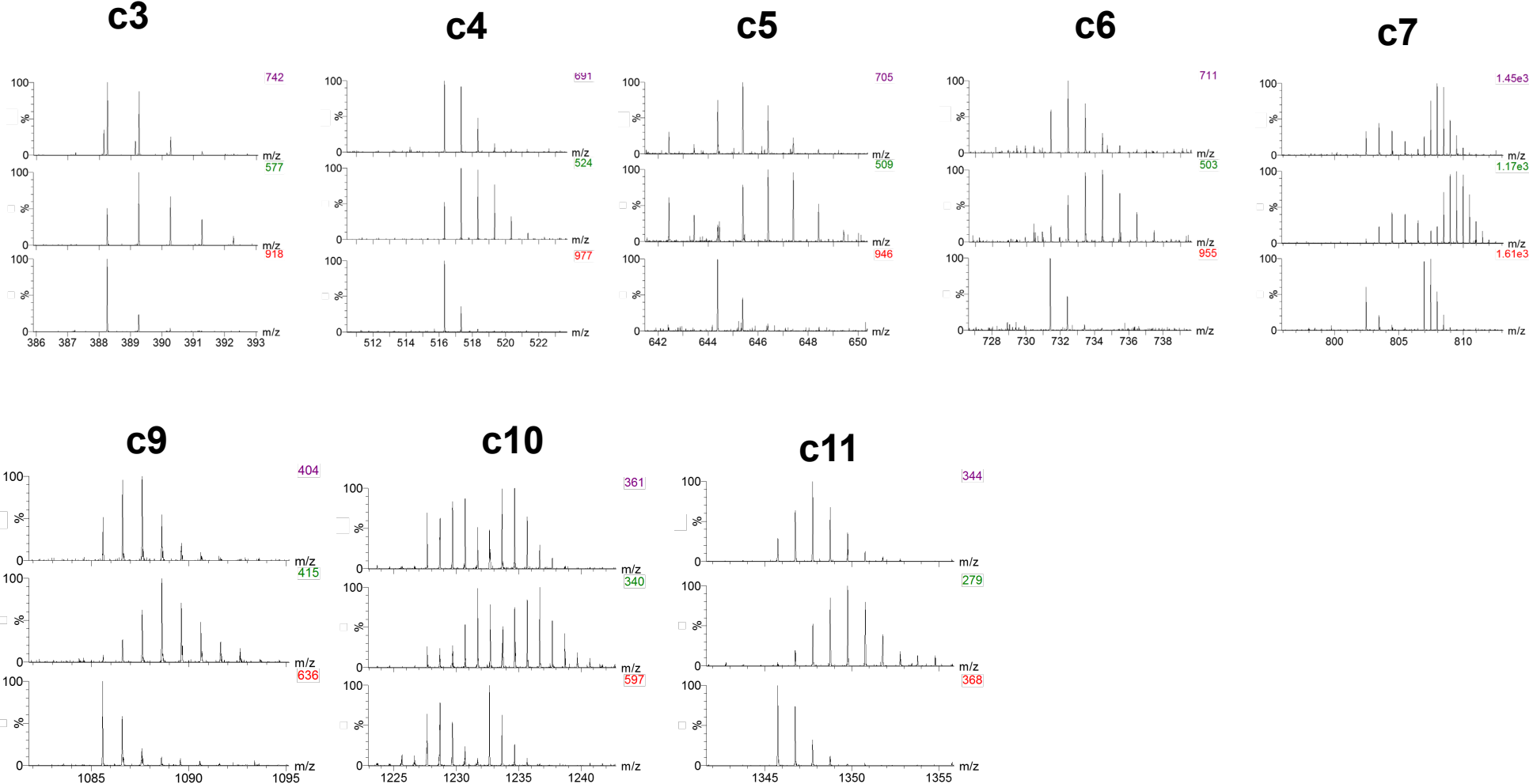
GCN5_100uM_ECD - TimePoint 0 seconds

240716_GCIN5_100uM_ECD_pos02_0sec_01 67 (8.034) Cm (52:80)

2: TOF MSMS 624.40ES+
1.09e6



MSMS – peptide IIKEPTDIL c ion series



With compound
Deuterated
ND

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

Newmarket **BETA: UNRELEASED SOFTWARE**

Curate data
Manually curate by adjusting processing parameters

Select a protein
GCN5

Show hidden peptides

Hide peptide

Sequence	Start	Length	Modifications
LEKQQS...LKPVSL	18	16	None
IIKEPTDIL	42	9	None

Items per page: 100 1 - 2 of 2

Apo+DDD4250
Apo

Relative Uptake (Da)

Exposure Time (minutes)

Timepoint: 0 sec

Select an overlay: Fractional uptake (%)

0 % 100 %

GHKEVQLKQDILGLVDLYLEKQQSAMPFLKPVSLSEAPDYDIKEPTDILLTRRRKARHGDKYTKEDFGIELKRFDFNCRLYNAPTTIZFYKYANLQTLIMPKYEAL

State: Apo Charge State: 3+

Unassign Accept new assignments Advanced curation

240913_GCN5_20uM_ToF_pos02_0sec_01, t = 0 sec
Show IMS 8 +0.02 Da peaks assigned

240913_GCN5_20uM_ToF_pos04_0sec_01, t = 0 sec
Show IMS 9 -0.02 Da peaks assigned

240913_GCN5_100uM_IMS_pos03_300sec_01, t = 5 min
Show IMS 14 +4.58 Da peaks assigned