

# An Introduction to Mass Spectrometry

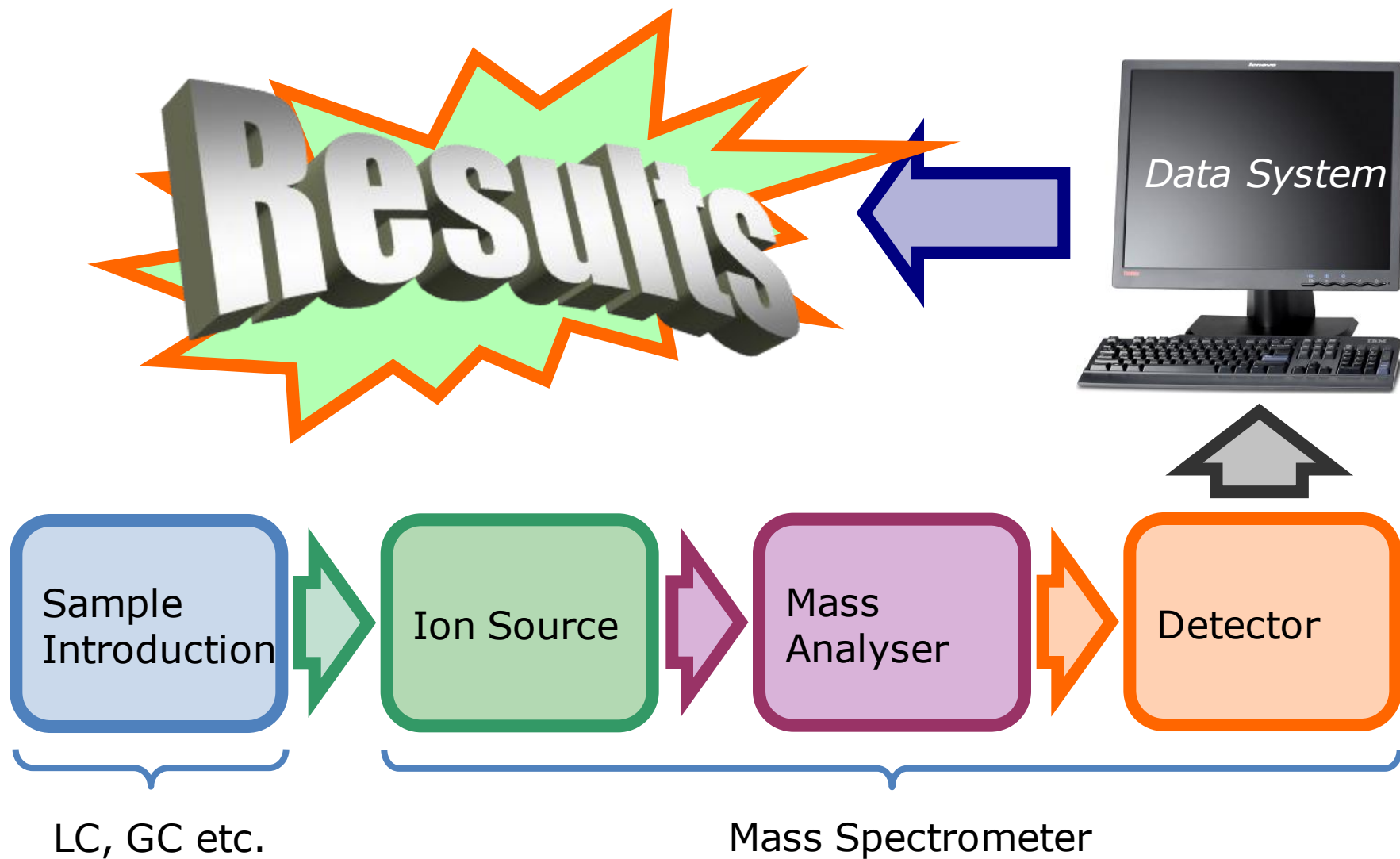


**Guy Wilson**

UPC<sup>2</sup> & Purification Business Development,  
Northern Europe, Waters Ltd



# What is a Mass Spectrometer?





- Atmospheric Pressure Ionisation (API)
  - A term for any ionisation process that is carried out at atmospheric pressure
  
- The most common modes of API are...
  - Electrospray Ionisation (ESI)
    - Most commonly used
  - Atmospheric Pressure Chemical Ionisation (APCI)
    - Less commonly used than ESI but still significant usage
  - Atmospheric Pressure Photo Ionisation (APPI)
    - A more niche ionisation mode for certain compounds that will not ionise by either ESI or APCI.

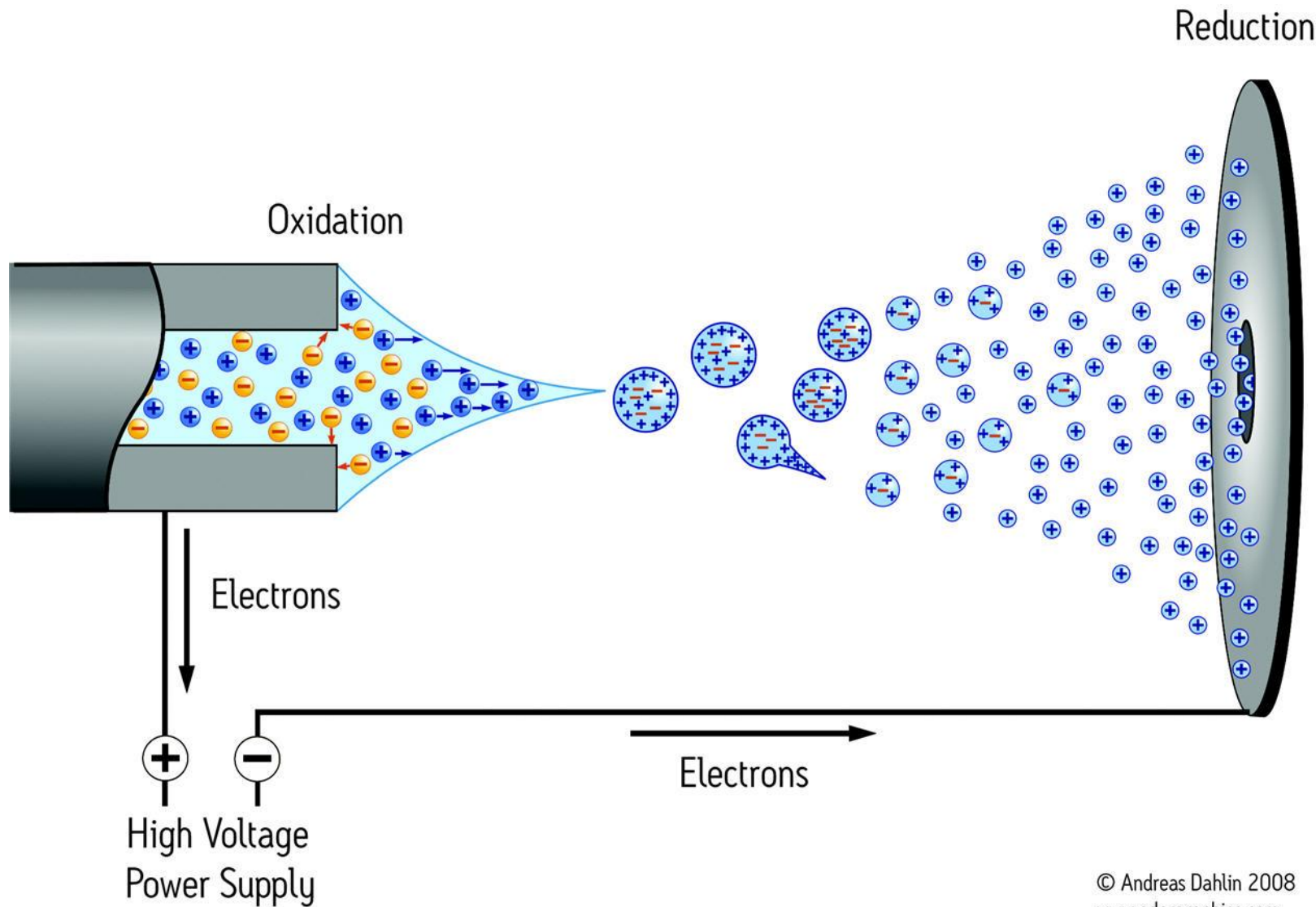


# Electrospray Ionisation (ESI)



# Electrospray Ionisation Overview

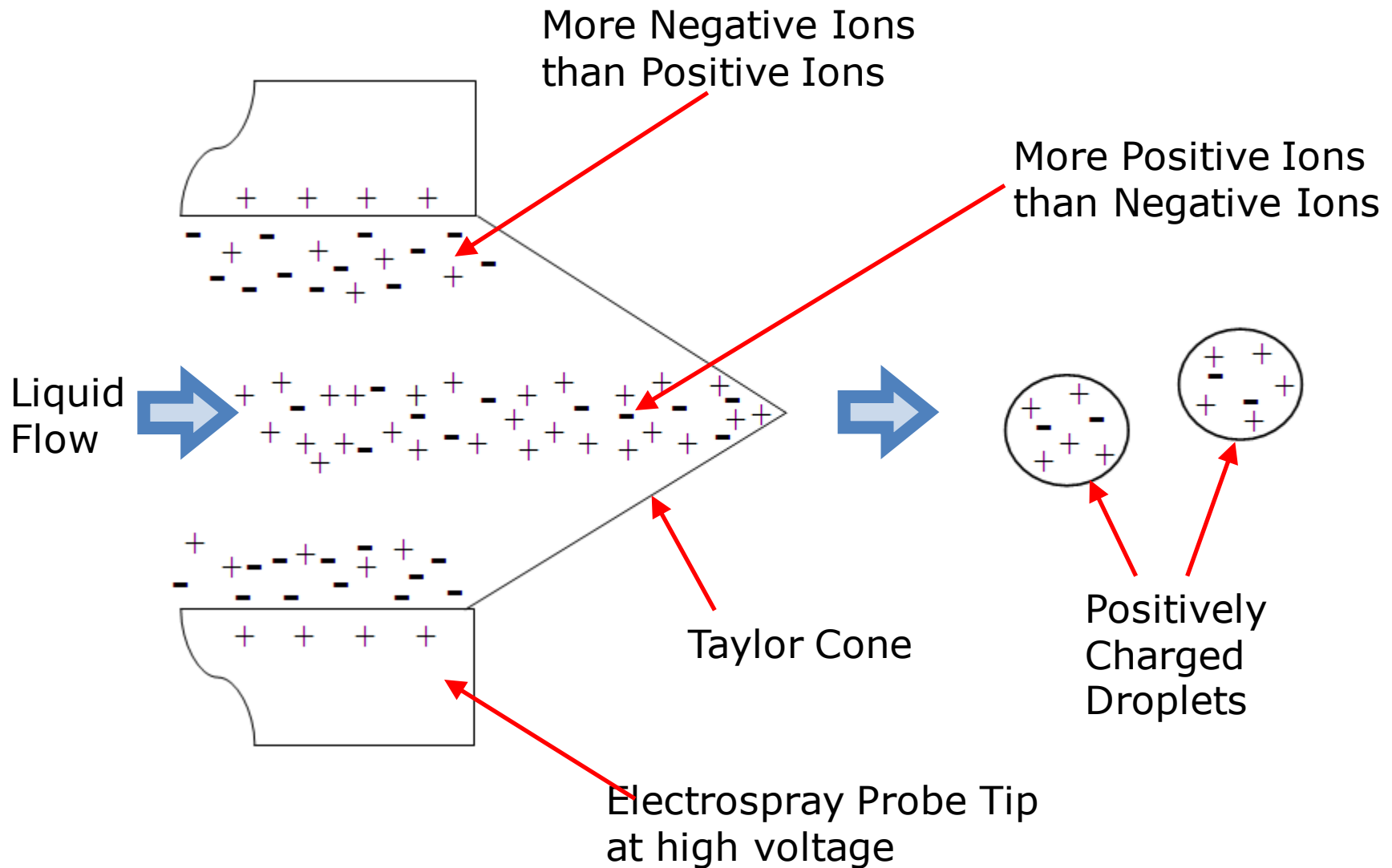
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# Electrospray Ionisation Producing Charged Droplets

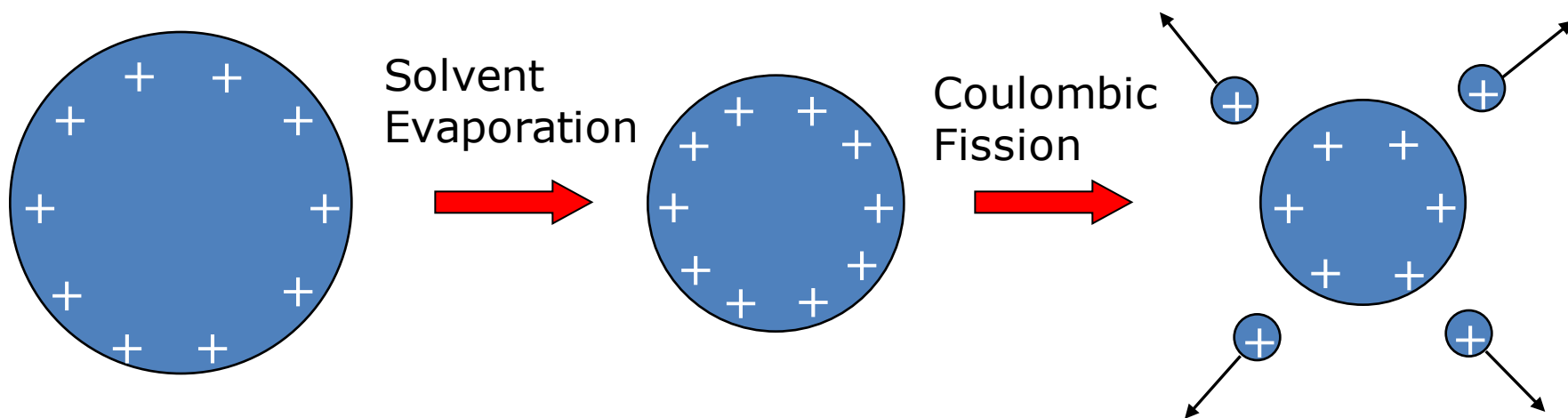




# Electrospray Ionisation

## Fission of Charged Droplets

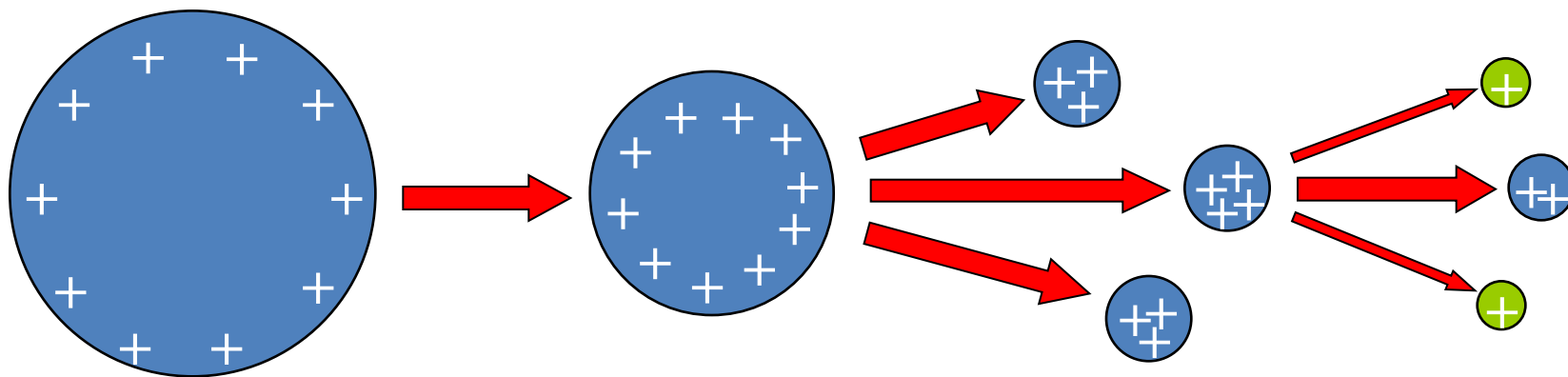
- Droplets produced from the spray have a surface charge
- Surface charged droplets undergo solvent evaporation and droplet fission to produce smaller droplets
- Like charge repulsion becomes greater than droplet surface tension and fission occurs to produce smaller charged droplets



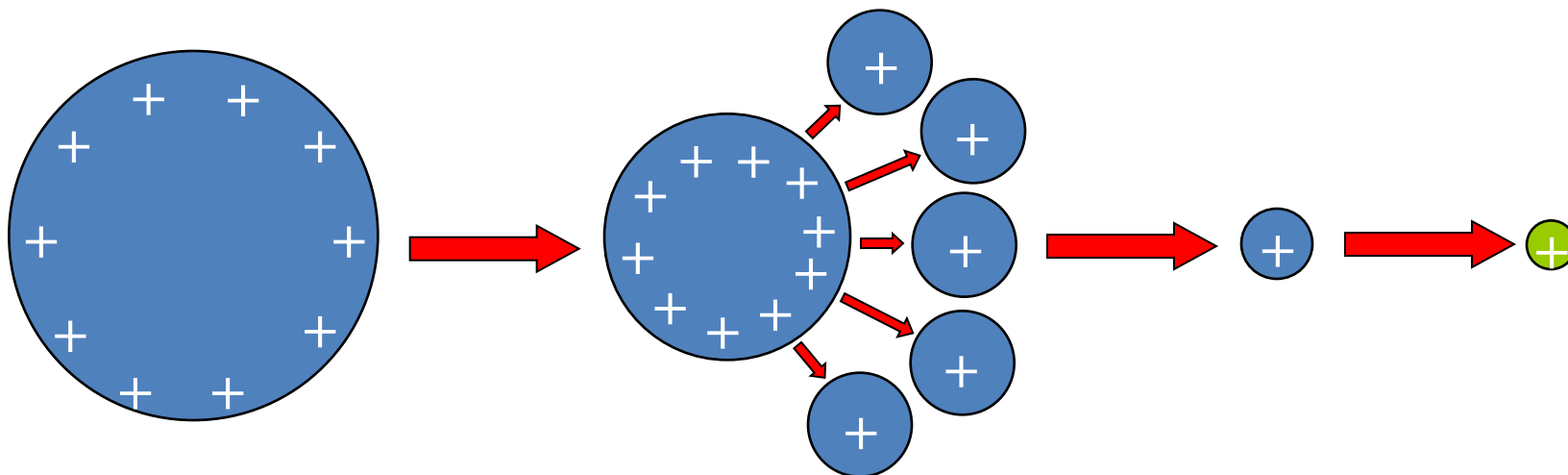


# Electrospray Ionisation Formation of Gas Phase Ions

## ■ Ion Desorption Mechanism



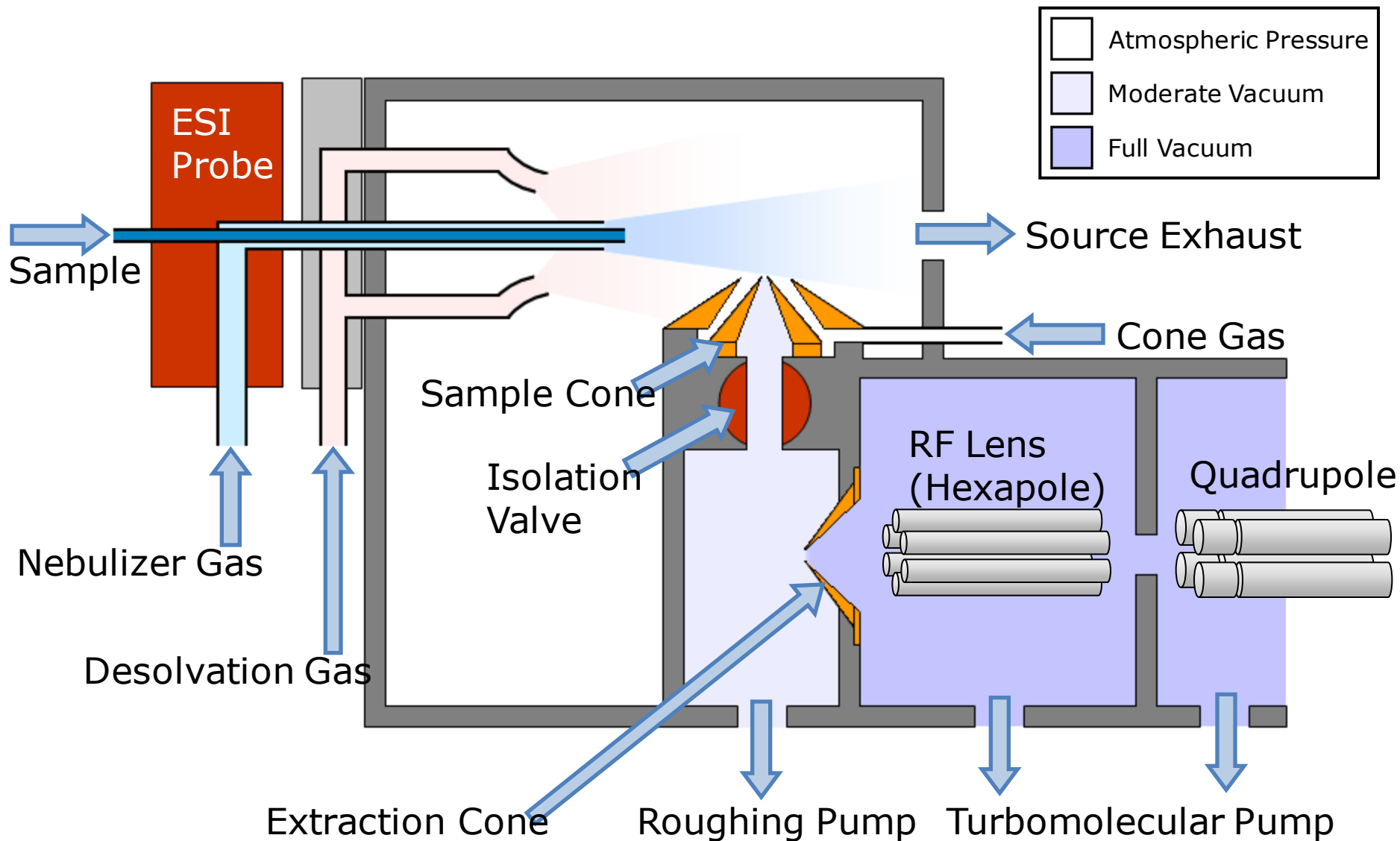
## ■ Charged Residue Mechanism





# Electrospray Ionisation Z-spray Ion Source

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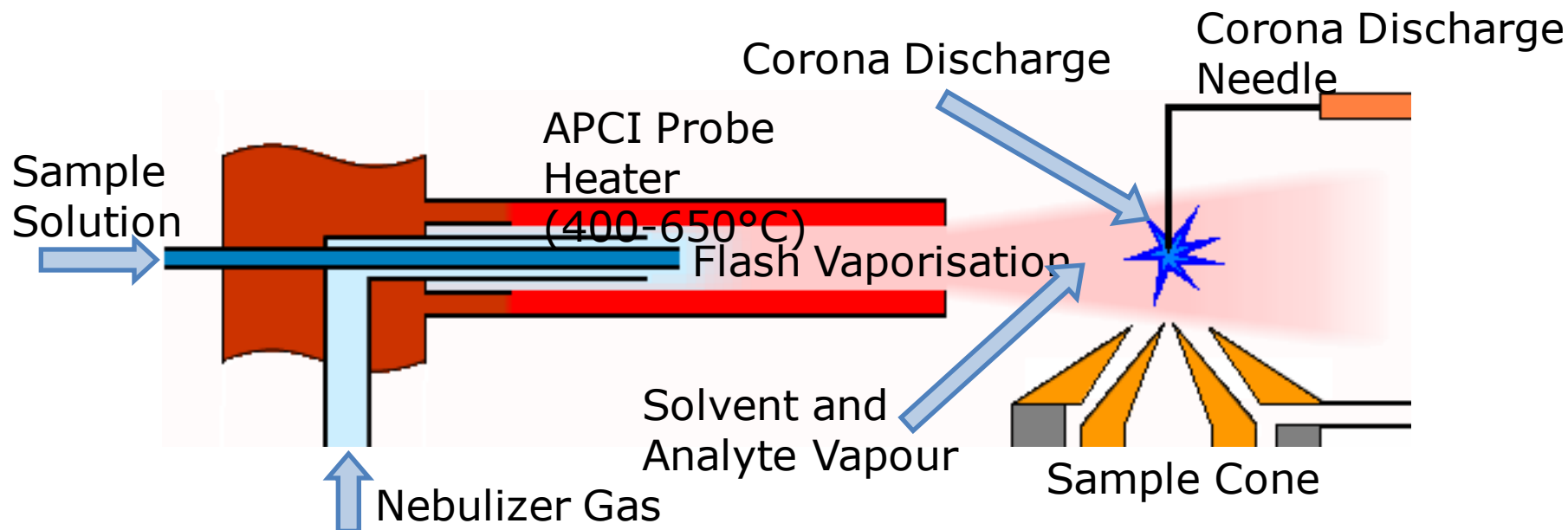


# Atmospheric Pressure Chemical Ionisation (APCI)



# APCI – Overview

- Liquid flow is forced through a narrow capillary to give it a high linear velocity
- The APCI Probe heater combined with nebulizer gas then vaporises the liquid flow
- The solvent and analyte vapour passes through the corona discharge region to produce gas phase ions



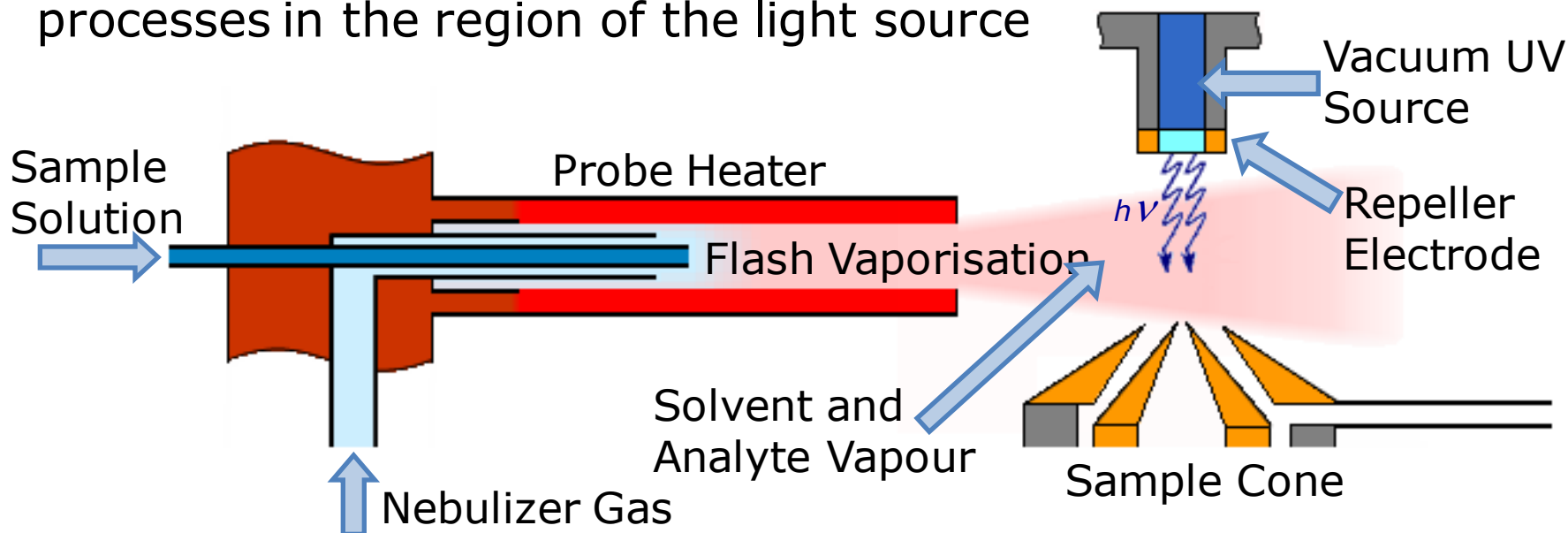


# Atmospheric Pressure Photo Ionisation (APPI)



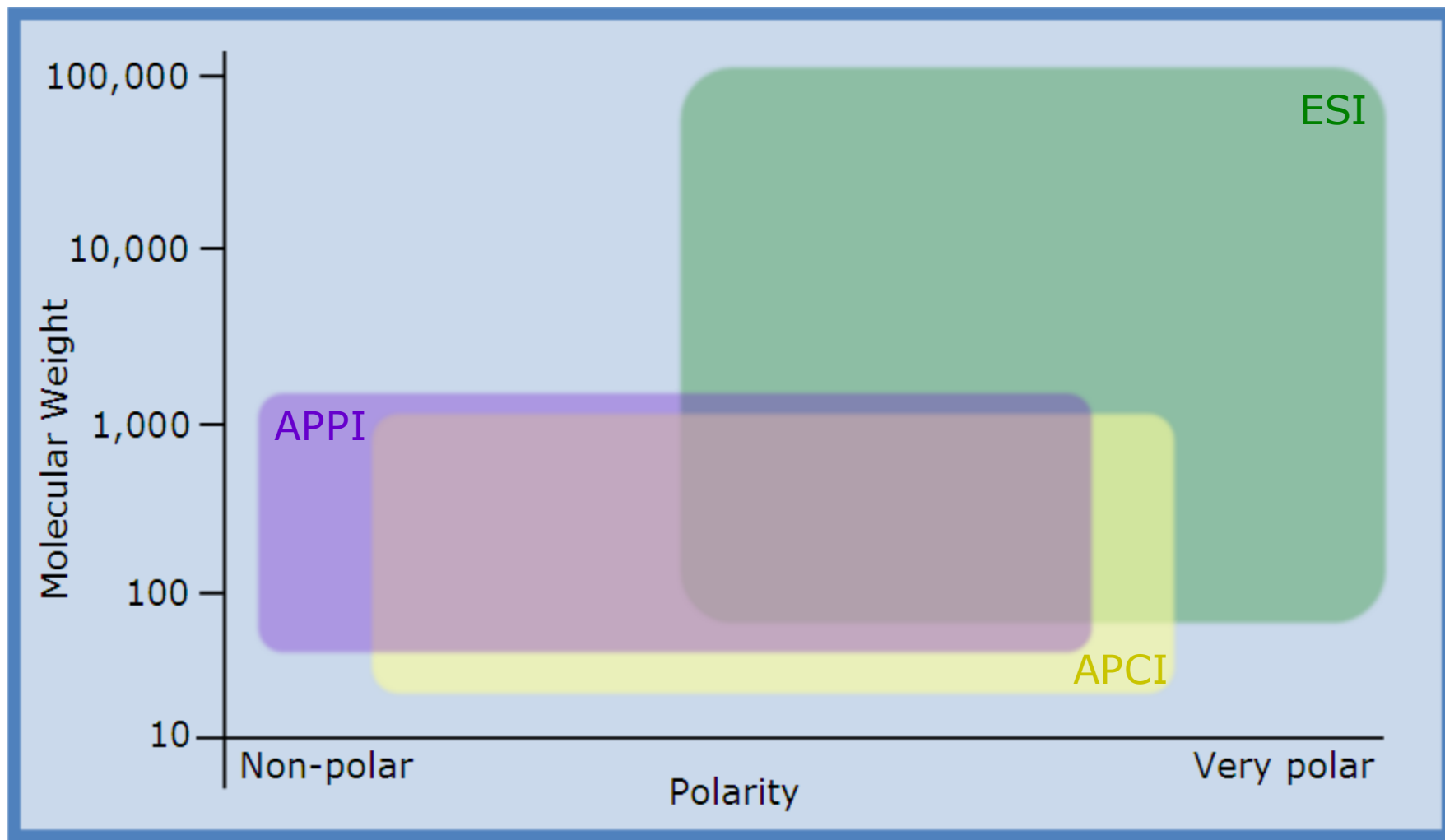
# APPI – Overview

- APPI uses the same probe as APCI with a Vacuum UV light source instead of the corona discharge needle
- As with APCI:
  - Liquid flow is forced through a capillary to give it a high linear velocity
  - The probe heater and nebulizer gas vaporise the liquid flow
- Ionisation occurs by either direct or chemical ionisation type processes in the region of the light source





# ESI / APCI / APPI Comparison





# Universal Source platform

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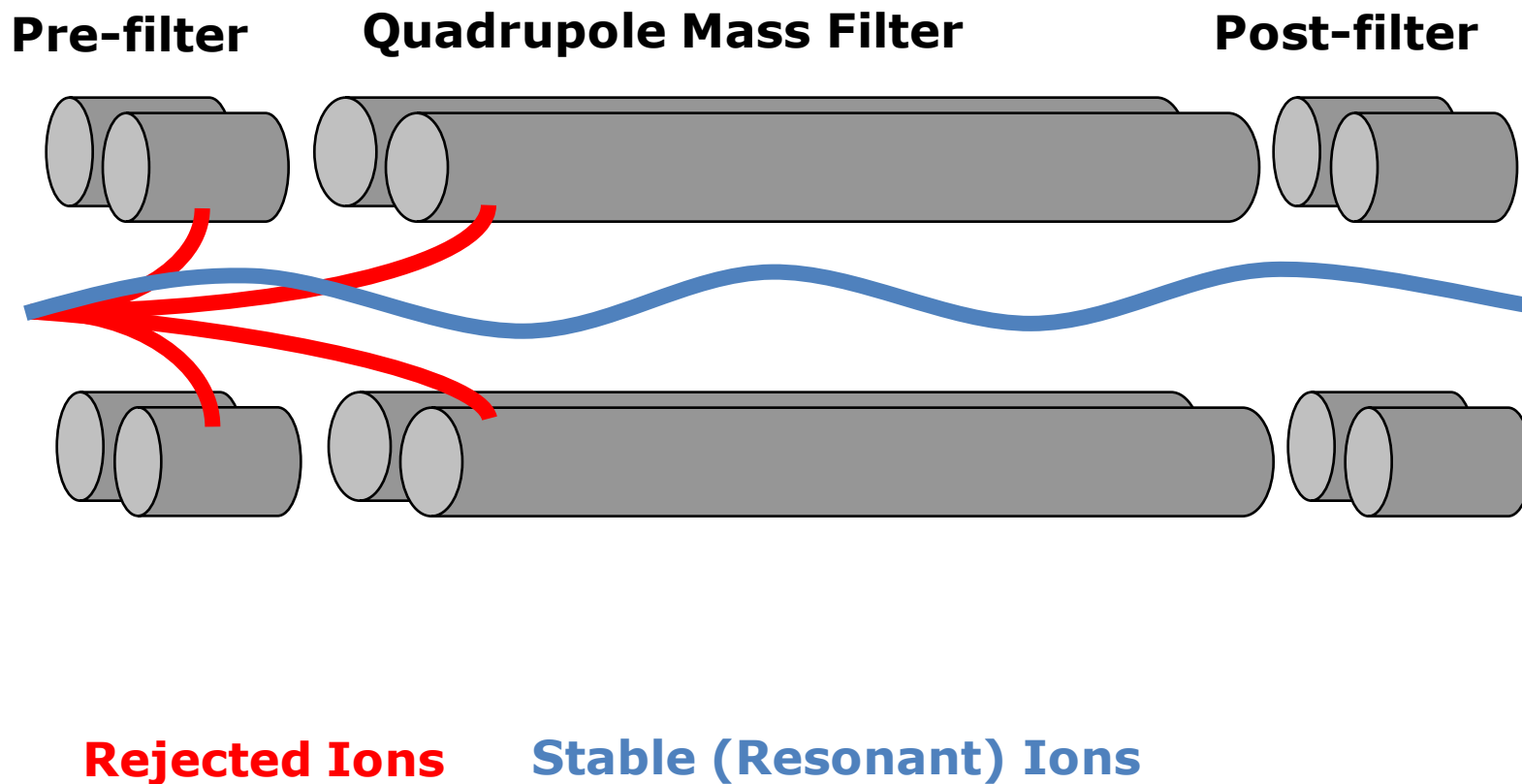
Also compatible with 3<sup>rd</sup> party source options:

*Phytronix **LDTD** — IonSense **DART**  
Prosolia **DESI** — Advion **NanoMate** — Protea **LAESI***



# Quadrupole Theory

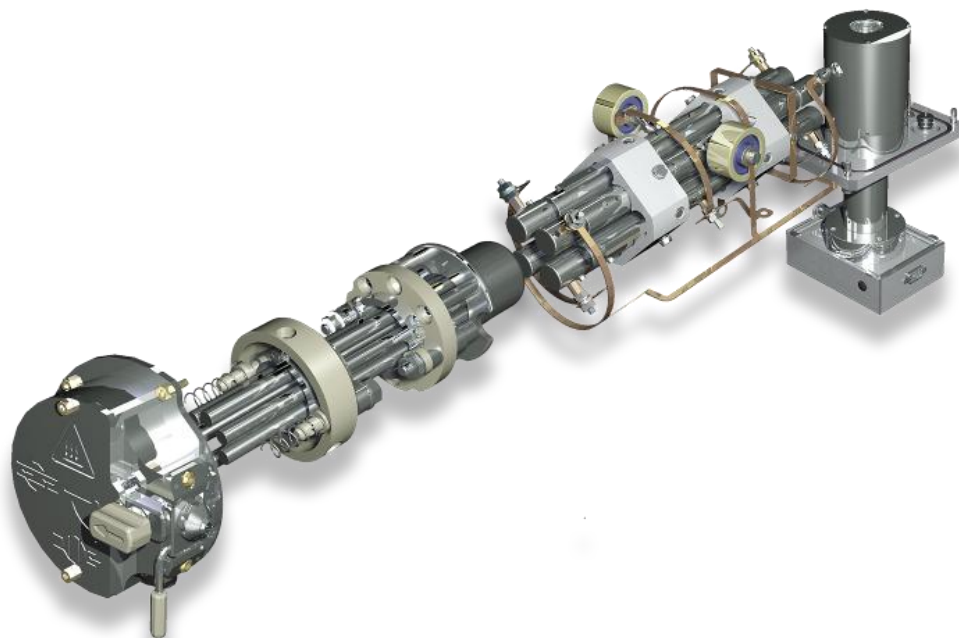
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# Single Quadrupole MS

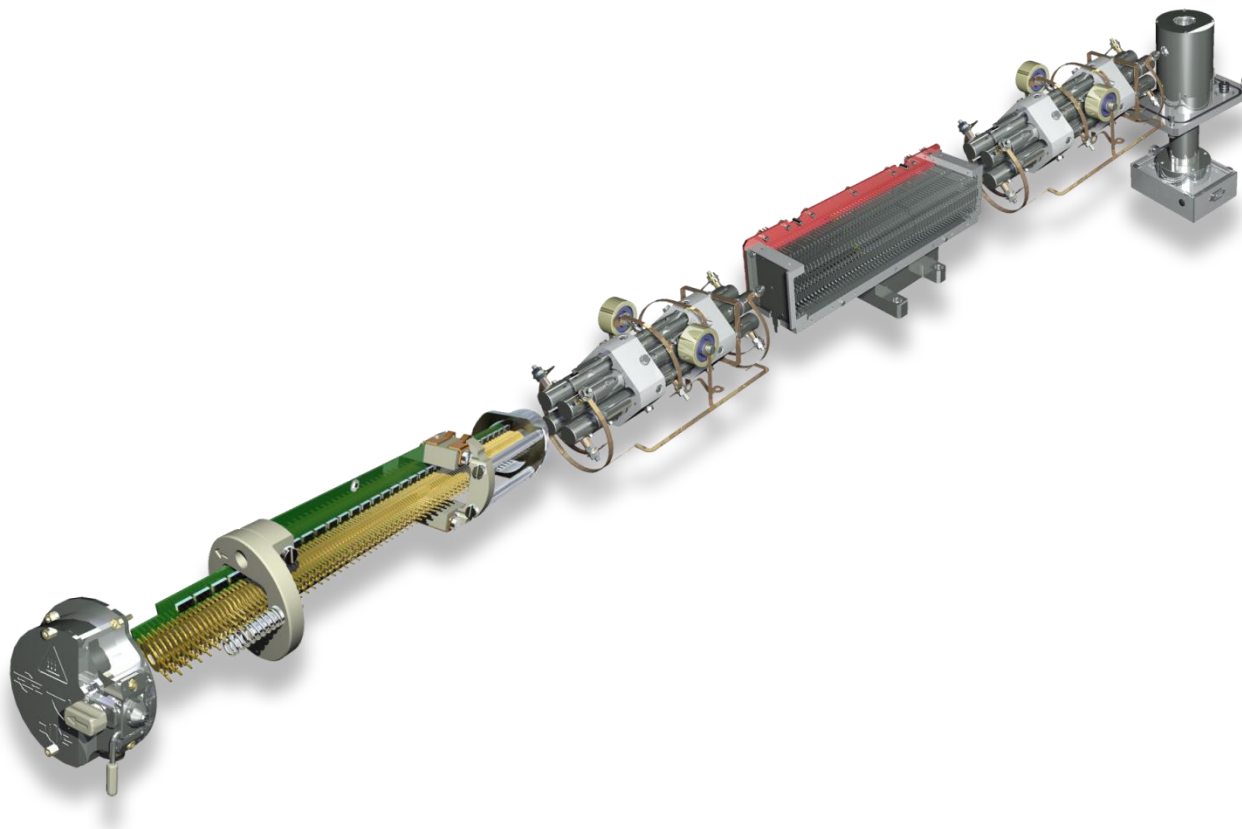
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# Tandem Quadrupole MS

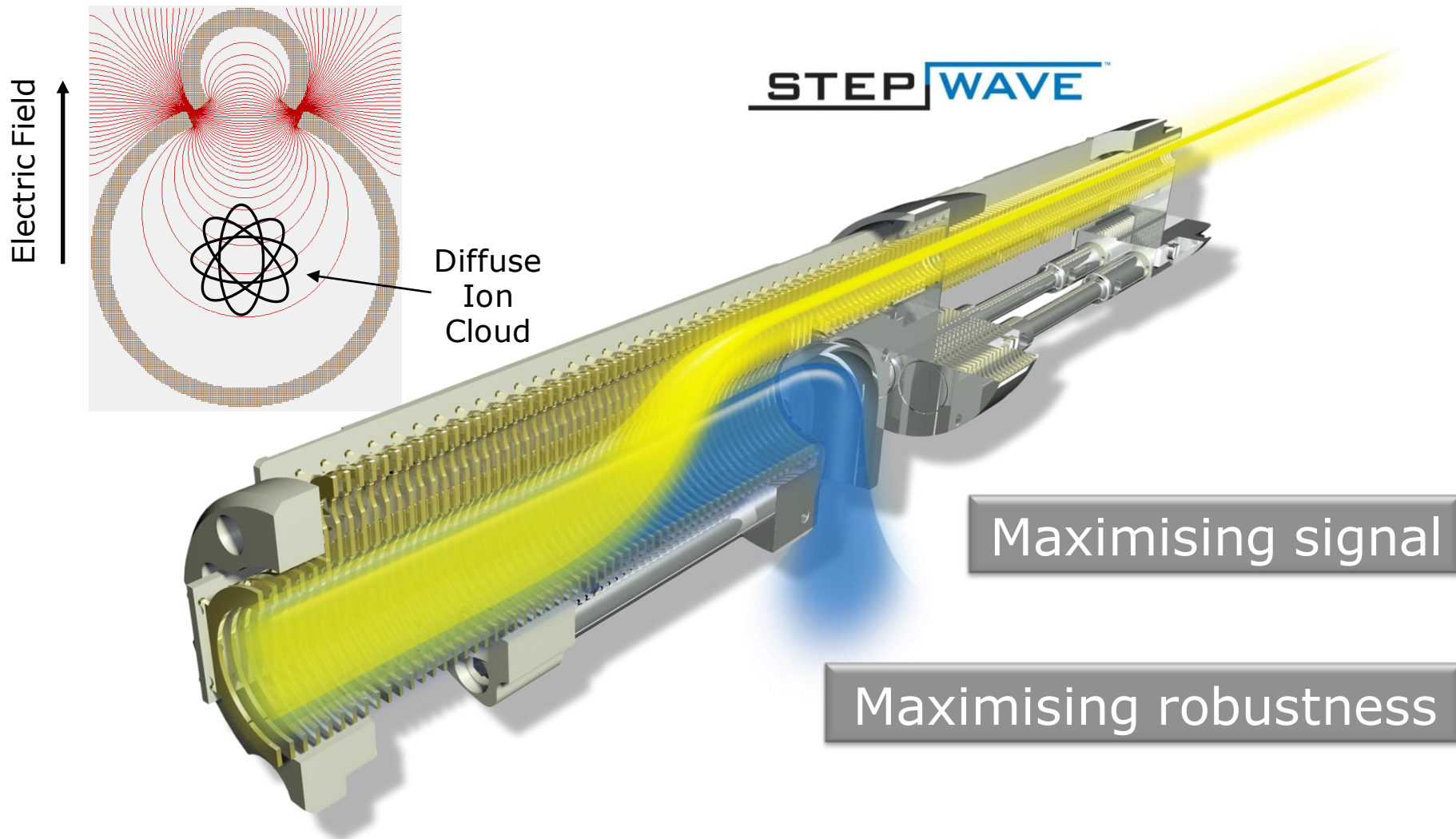
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# Transfer Region Ion Optics

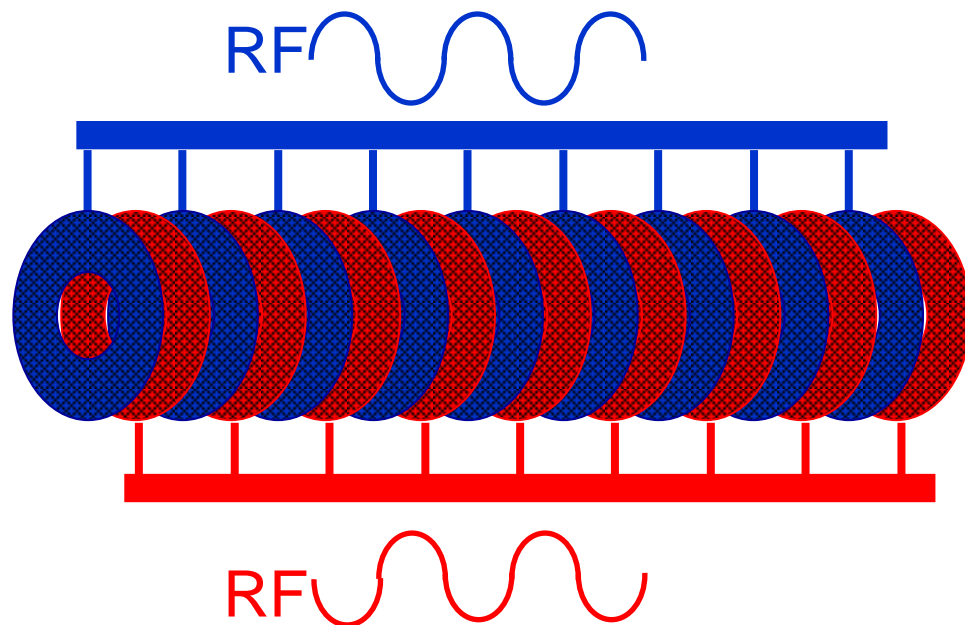
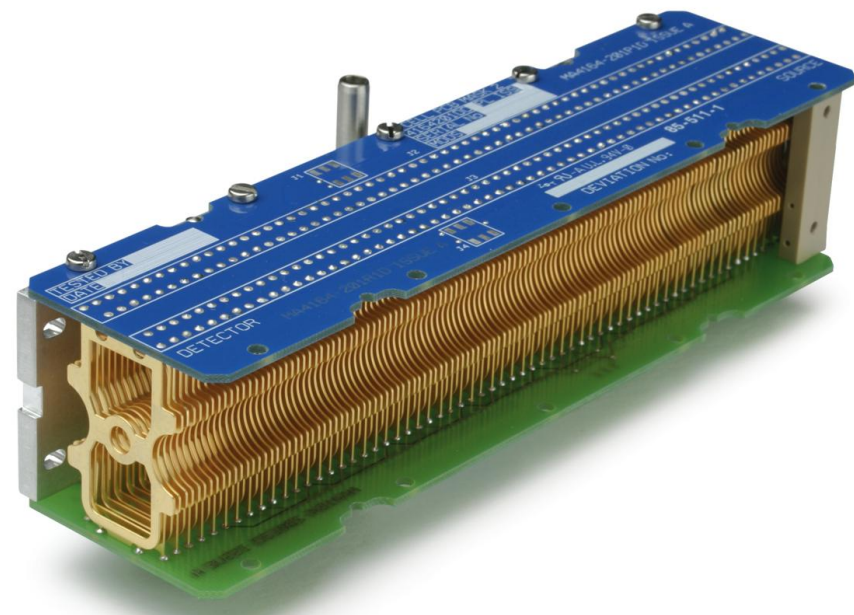
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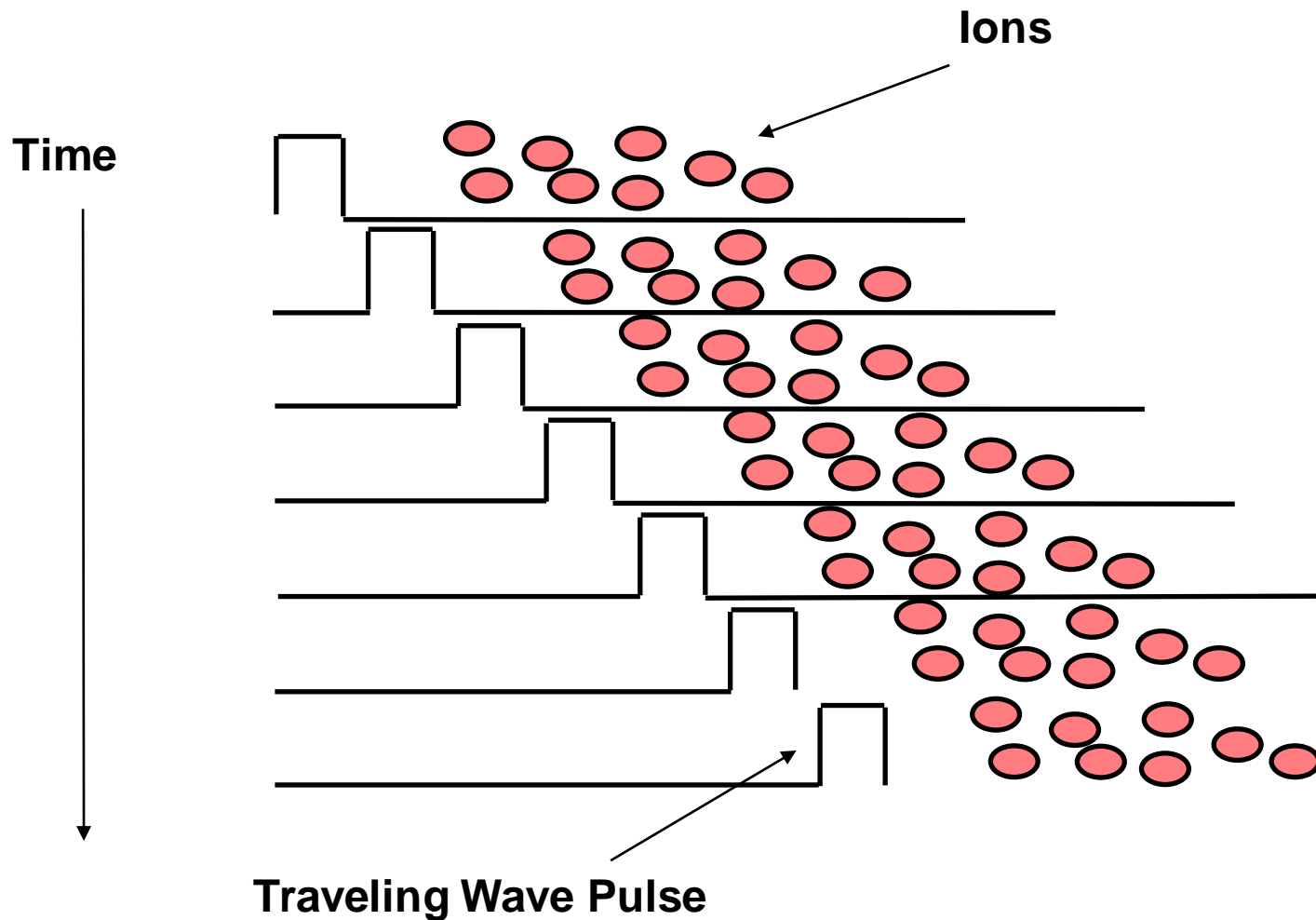
# Traveling Wave Ion Transport

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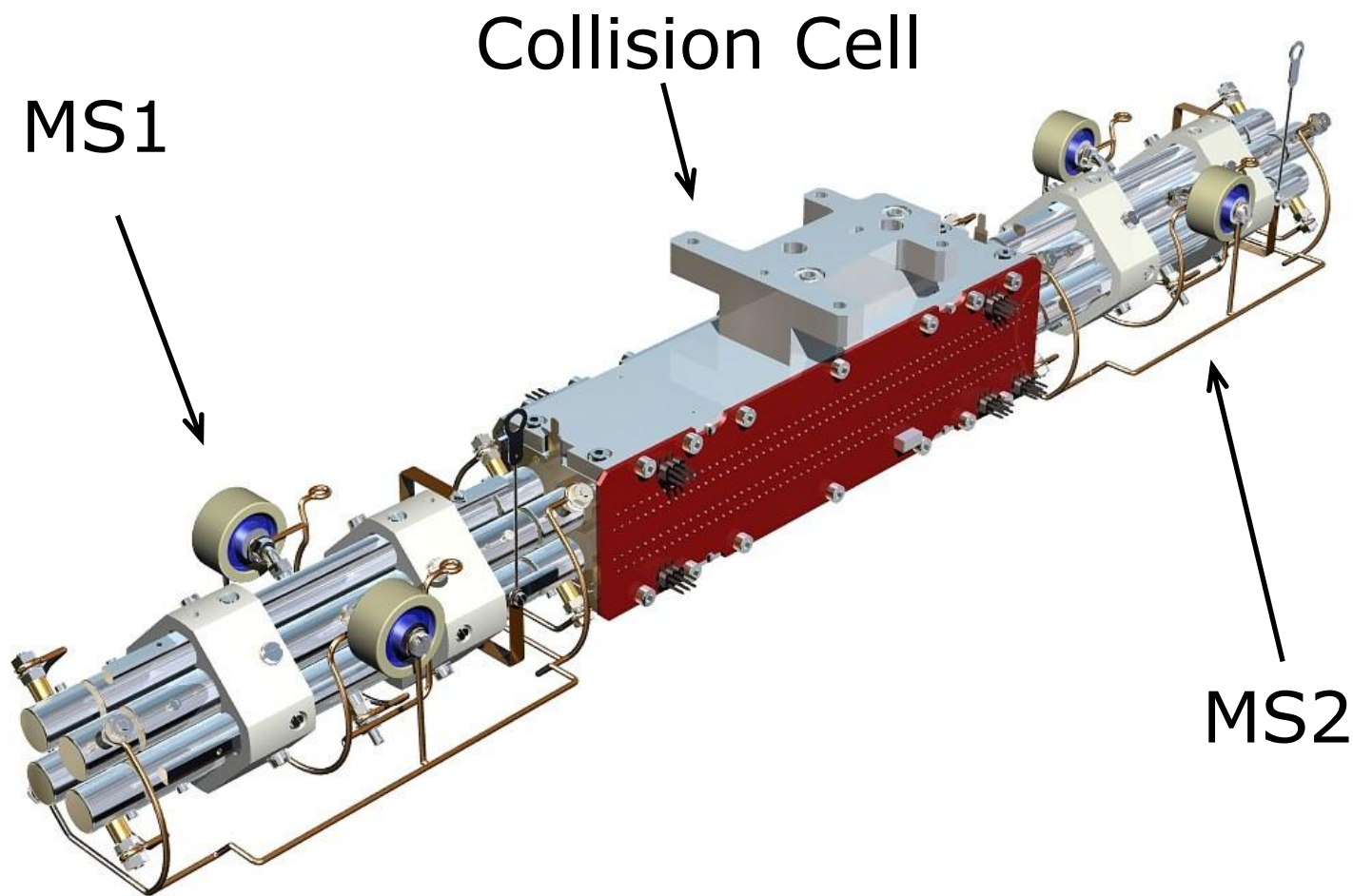


# Traveling Wave Ion Transport



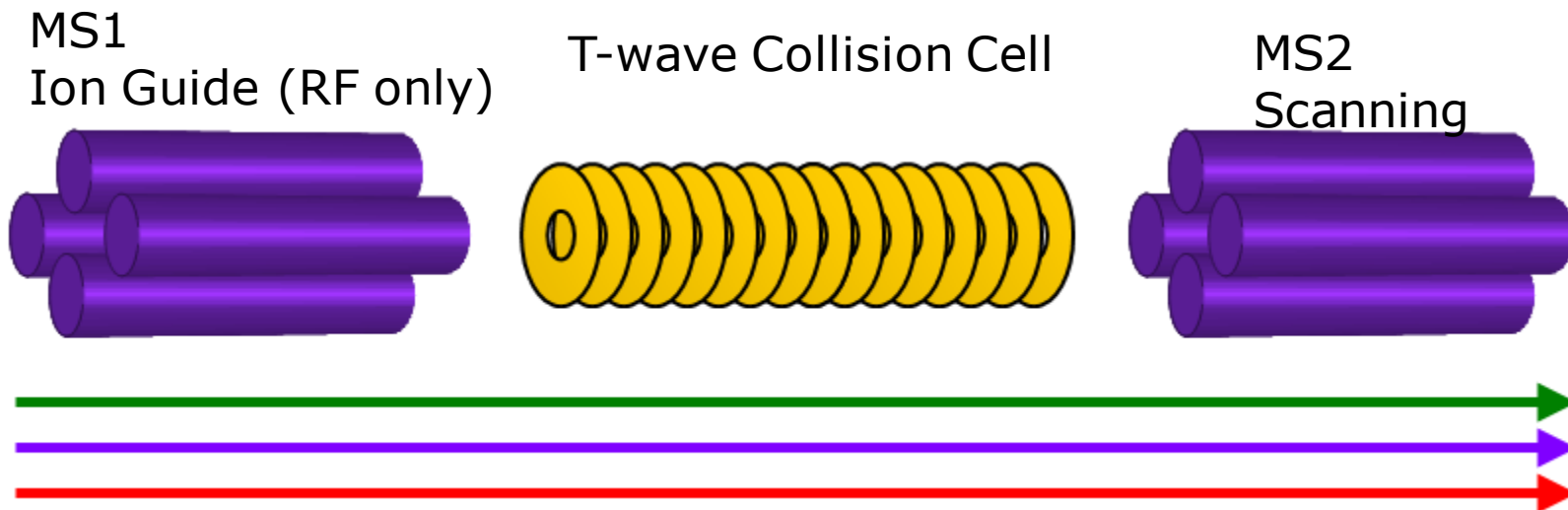


# Different Types of Experiments





# MS2 Scan



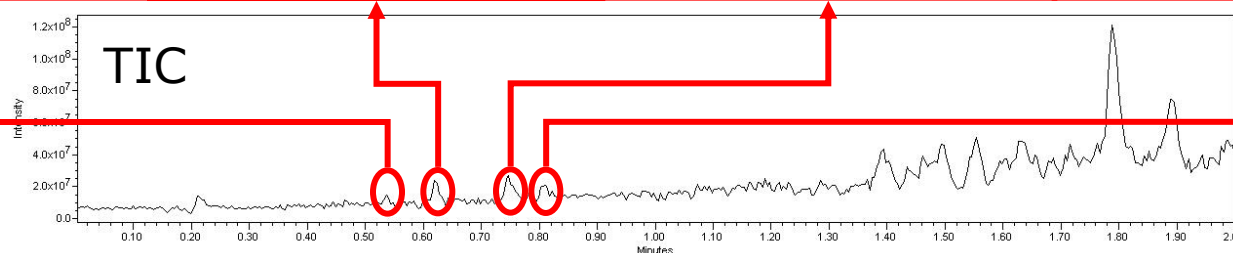
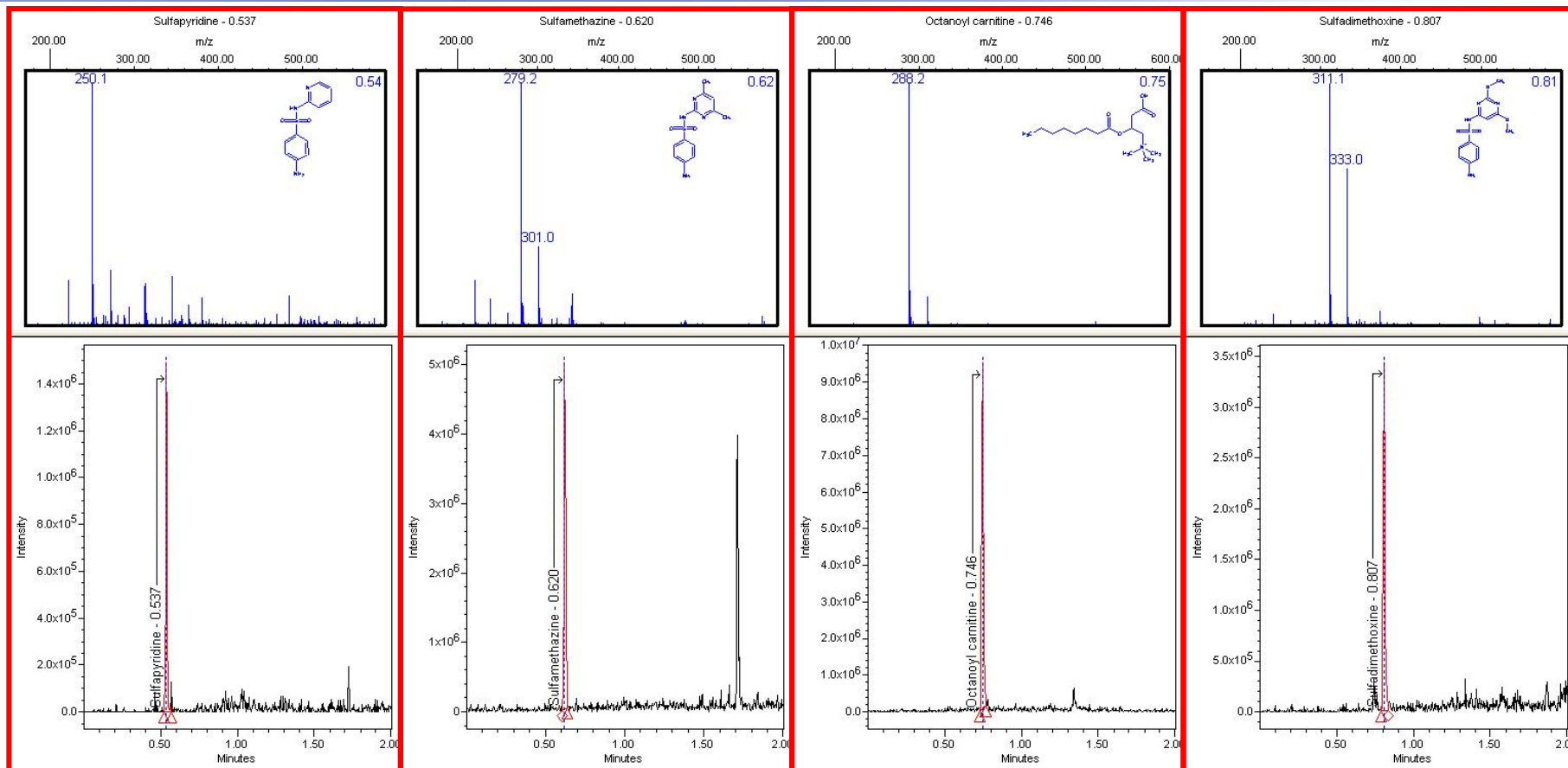
- Works in the same way as MS Scan except:
  - MS1 and Collision Cell act only as Ion guides
  - MS2 is the scanning quadrupole
- Minimises distance and amount of ion optics to interact with between mass resolved ions and the detector
- Better performance for fast scanning ( $> 2,000$  Da/sec)



# MS Scan - Example

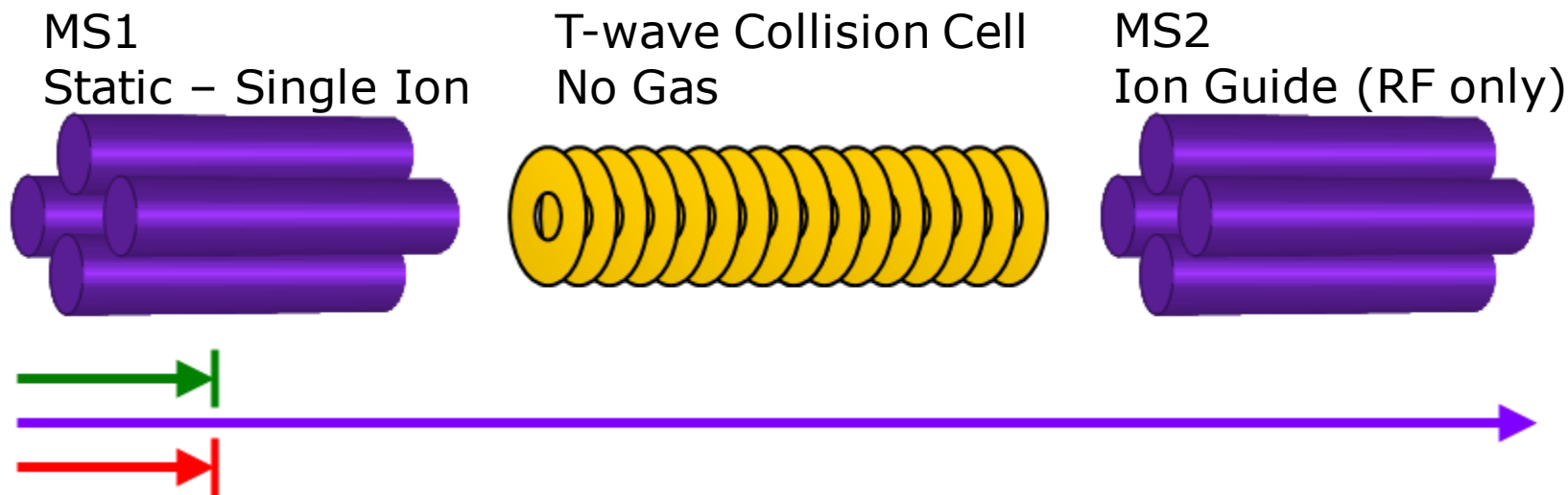
Mass Spectrum

Extracted Mass Chrom





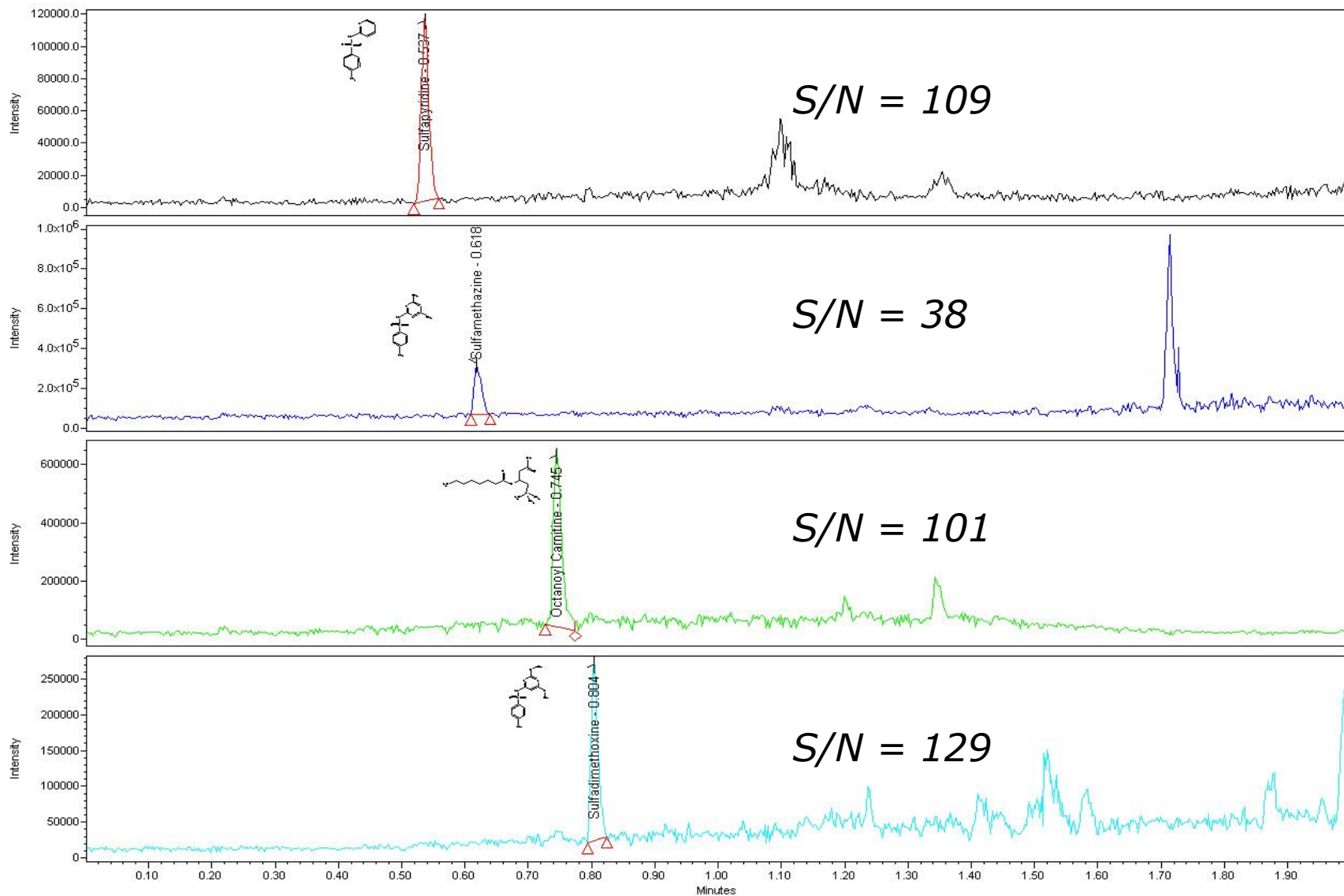
# SIR – Single Ion Recording



- Only selected ions are transmitted through the instrument and are monitored constantly
- Due to the instrument not acquiring other ions considerably more time is spent on each selected ion making this a much more sensitive acquisition mode than scanning acquisition modes
- No mass spectra are produced for this type of acquisition

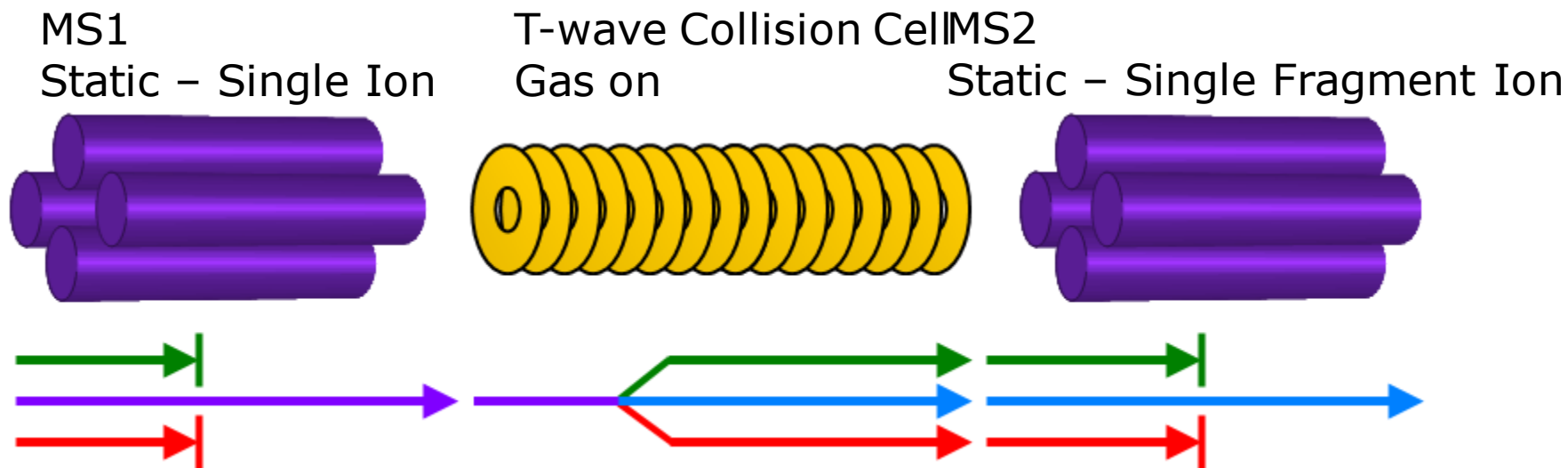


# SIR - Example





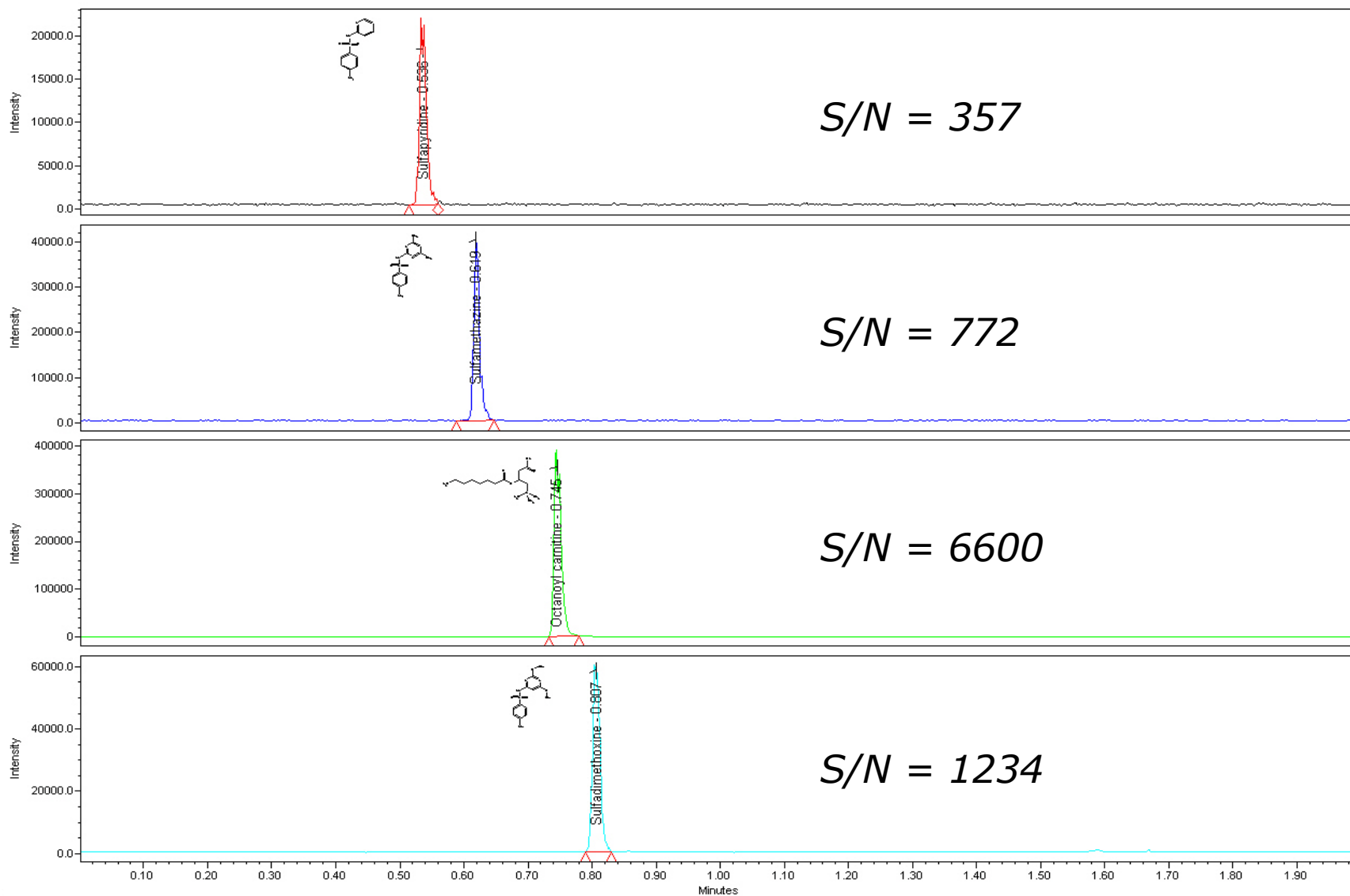
# MRM – Multiple Reaction Monitoring



- A selected ion is transmitted through the first quadrupole (precursor ion), fragmented in the collision cell, and a specified fragment ion is then transmitted through the second quadrupole (product ion).
- More selective and sensitive than SIR due to
  - Specific transition needed for response to be seen
  - Less interference by background ions of the same mass
- No mass spectra are generated by MRM experiments



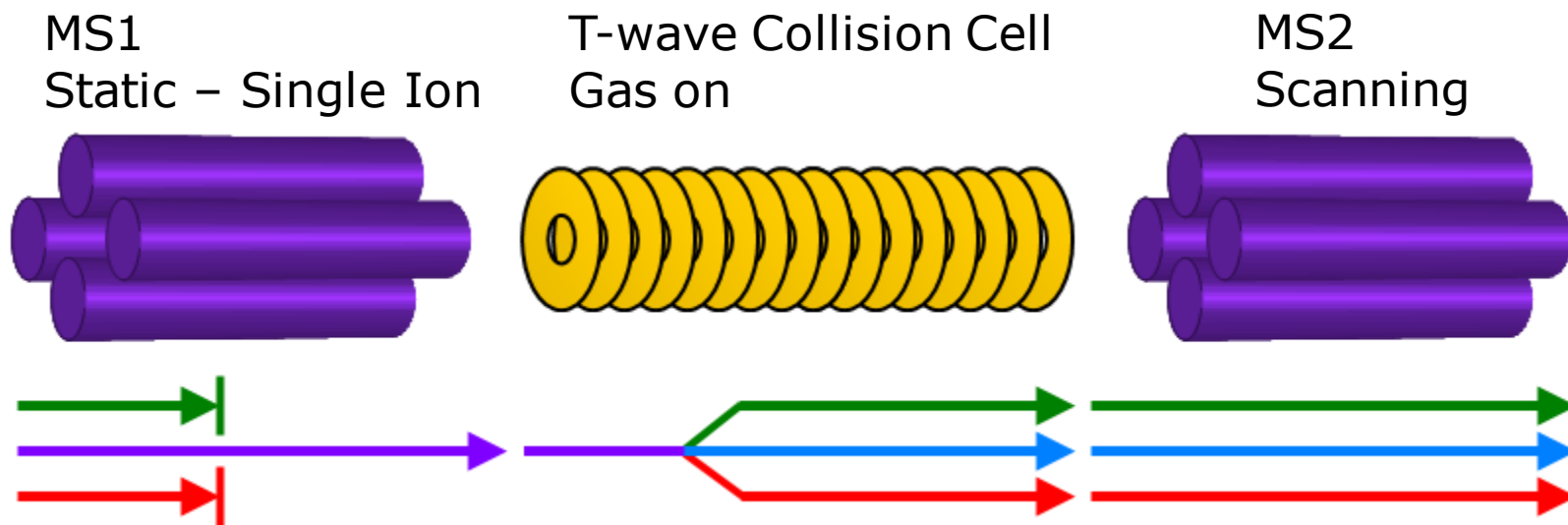
# MRM - Example



10ng/mL of each component



# Product Ion Scanning

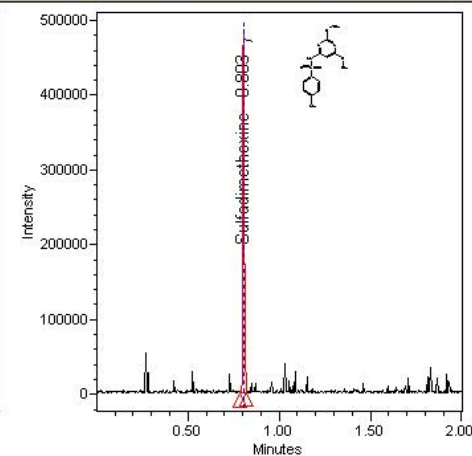
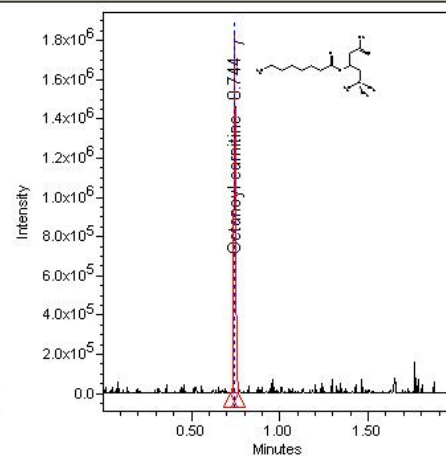
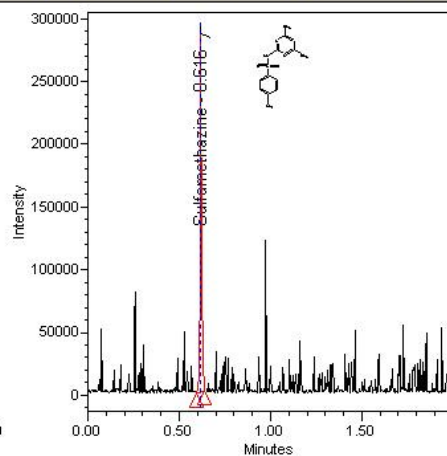
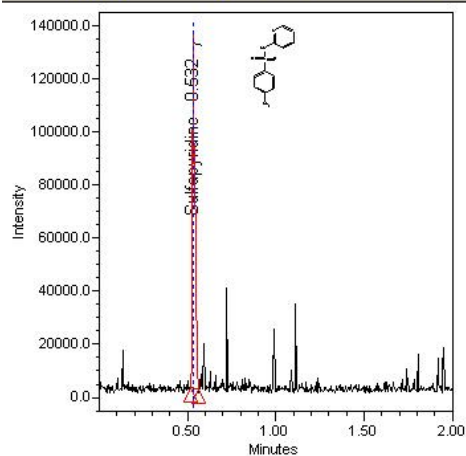
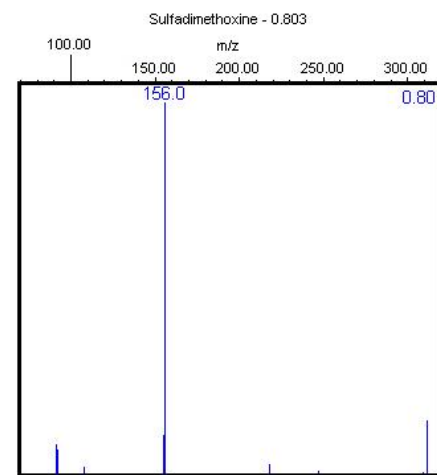
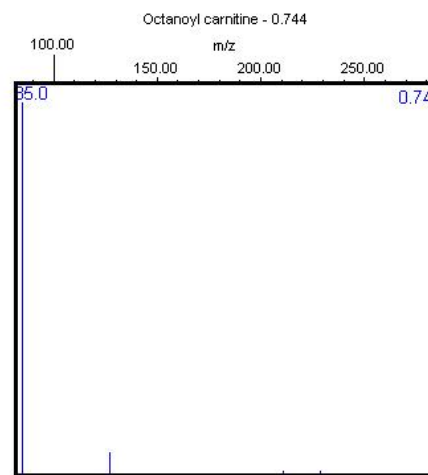
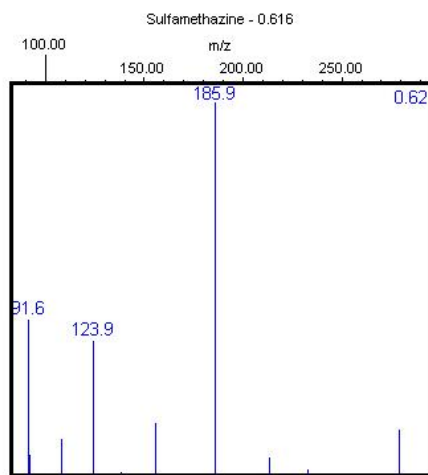
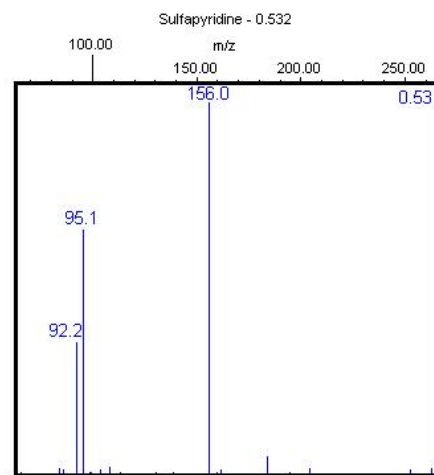


- Selected ions are transmitted through the first quadrupole (precursor ions) and fragmented in the collision cell
- MS2 is then scanned over a user defined mass range
- A mass spectrum of the product ions generated by fragmentation is acquired at each time point throughout the acquisition.



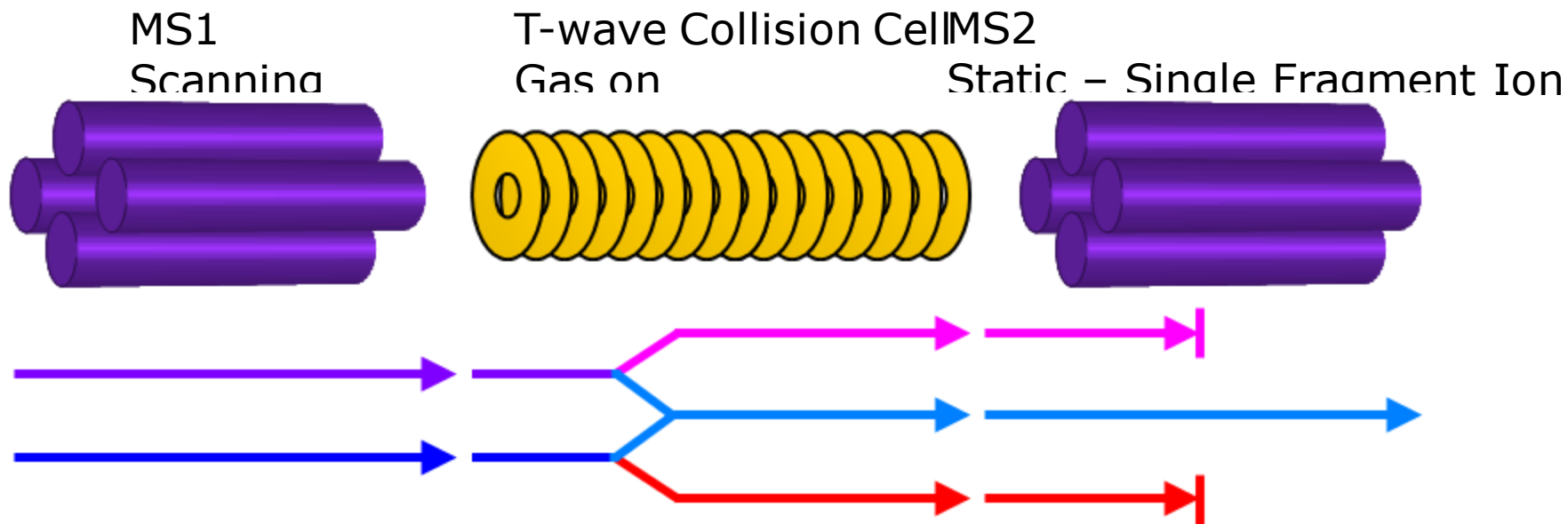
# Product Ion Scanning - Example

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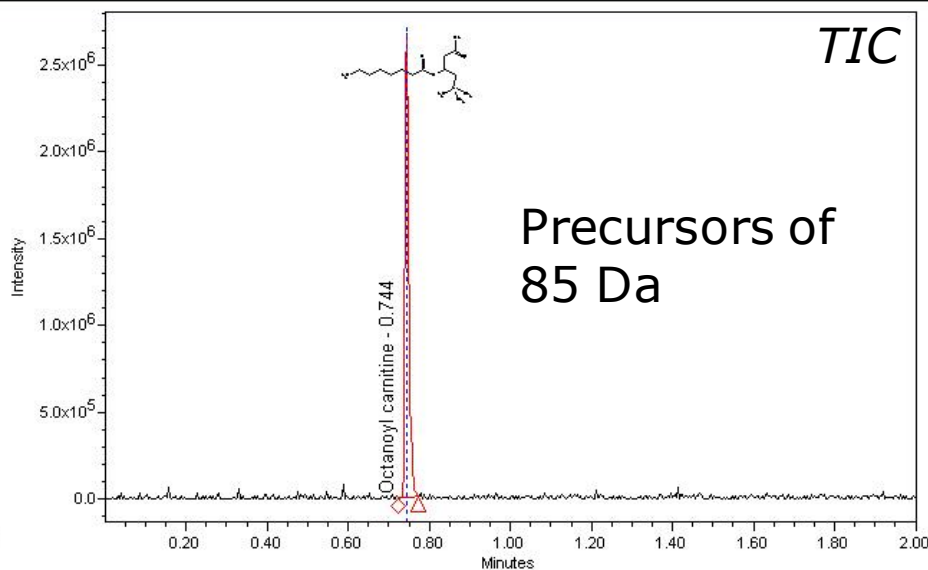
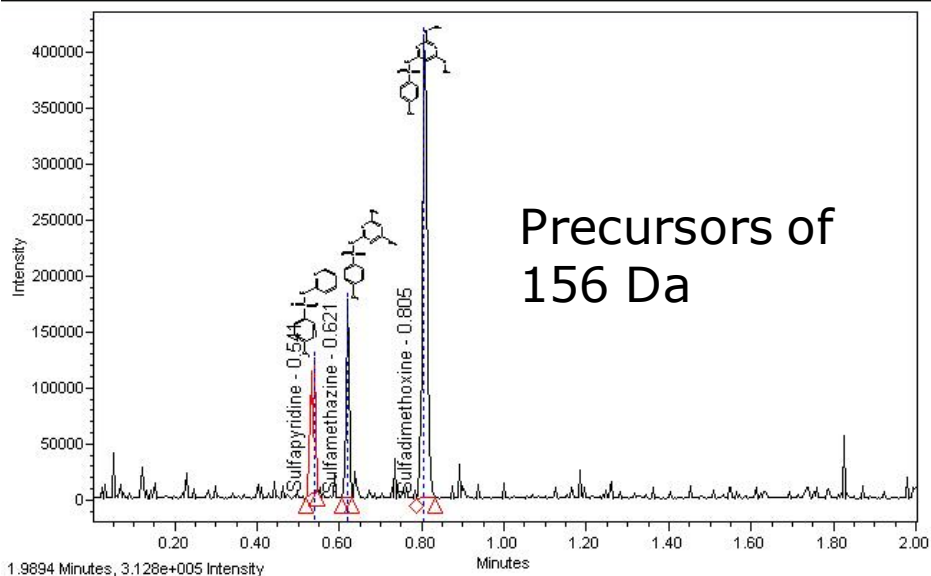
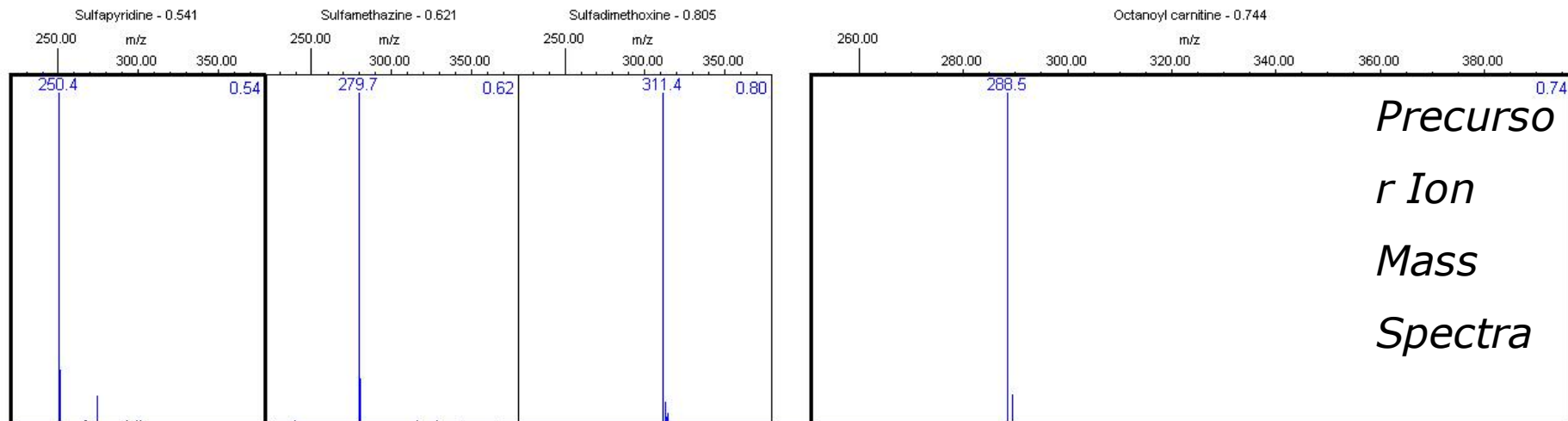
# Precursor Ion Scanning



- MS1 is scanned over a specified mass range and all ions are sequentially passed through to the collision cell where they are fragmented
- MS2 is set to transmit only the mass of a specific fragment ion and does not scan
- Any ions that fragment to give the specified product ion will generate a result.

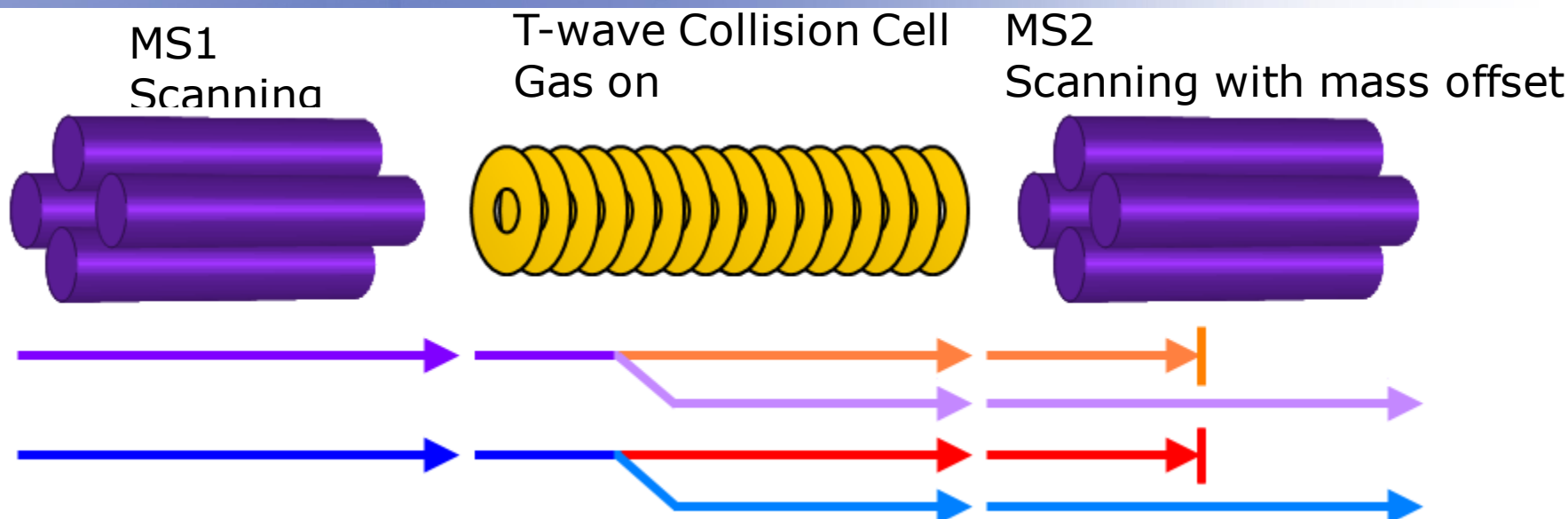


# Precursor Ion Scanning - Example





# Constant Neutral Loss



- MS1 is scanned over a specified mass range and all ions are sequentially passed through to the collision cell where they are fragmented
- MS2 is scanned in sync with MS1 over the same mass range minus an offset
- A response is only seen if a precursor ion loses a neutral fragment in the collision cell of the same mass to charge ratio as the offset between MS1 and MS2



# Waters Quadrupole Mass Spectrometers

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**SQD 2**



**Xevo TQD**

**Xevo TQ-S micro**



**Xevo TQ-S**





- Time-of-flight mass spectrometry (Tof-MS) is probably the simplest method of mass measurement to conceptualise, although there are hidden complexities when it comes to higher resolution instruments.
- The inherent characteristics of Tof MS are extreme sensitivity (all ions are detected), almost unlimited mass range, speed of analysis (modern instruments can obtain >10 full spectra per second) and sub 5ppm mass accuracy.



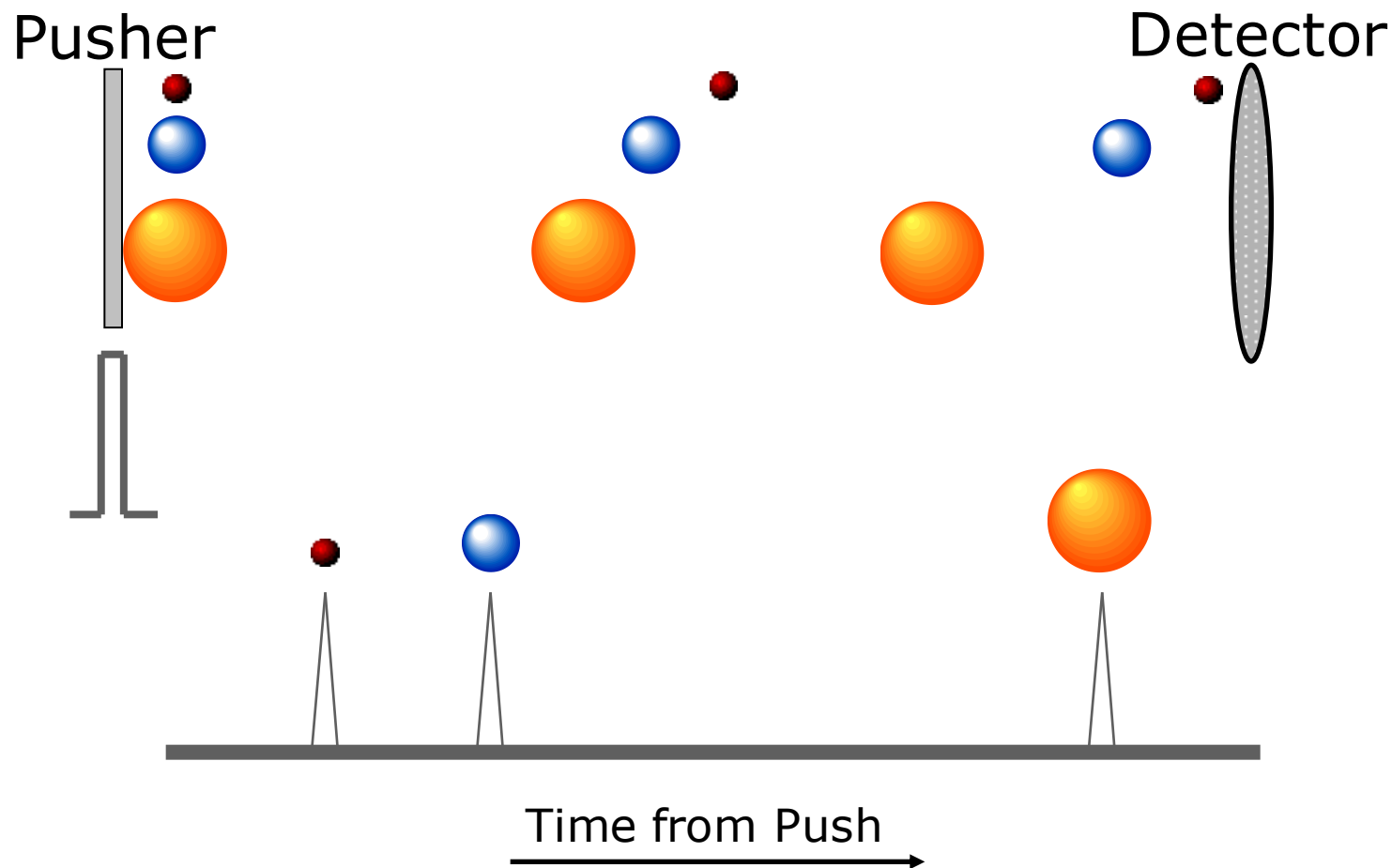
# Time Of Flight Theory

- A voltage pulse applied to a pusher plate (typically, 800-1000V) initiates an orthogonal acceleration TOF (oa-TOF)  $m/z$  measurement
- The energy imparted by this pulse in combination with an electric field is converted into kinetic energy (KE)
- The time it takes an ion to travel (TOF) a given distance ( $d$ ) is proportional to its mass-to-charge ratio or  $m/z$



# Time Of Flight Theory

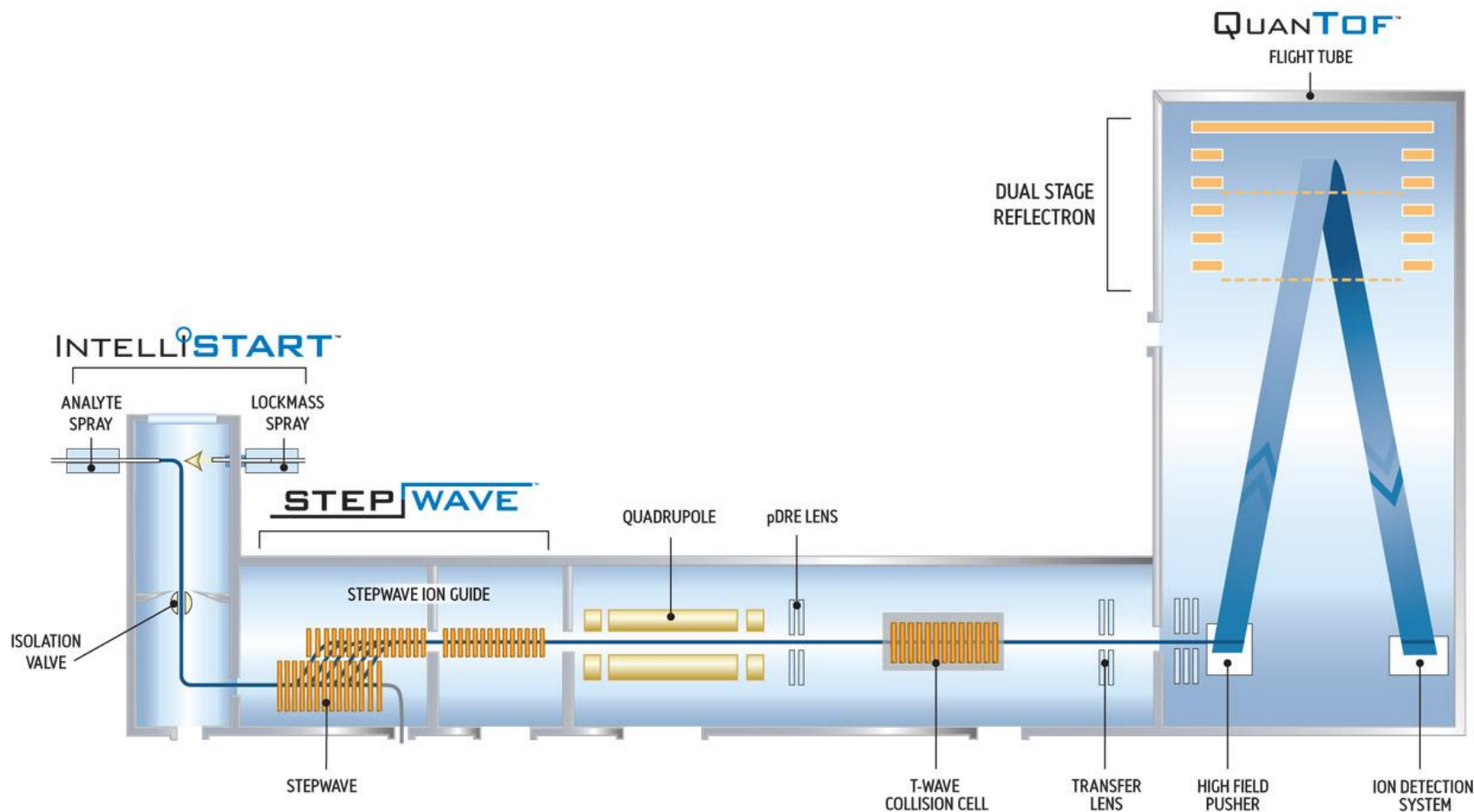
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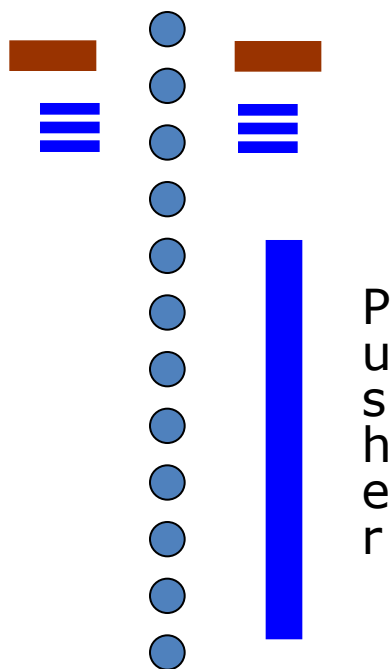
# Maximising Resolution

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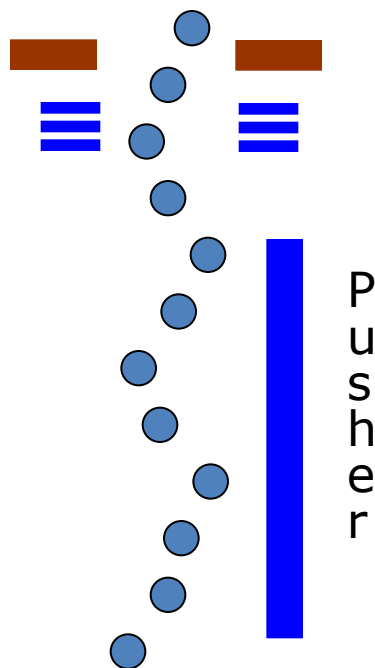


# Accelerating the Ions



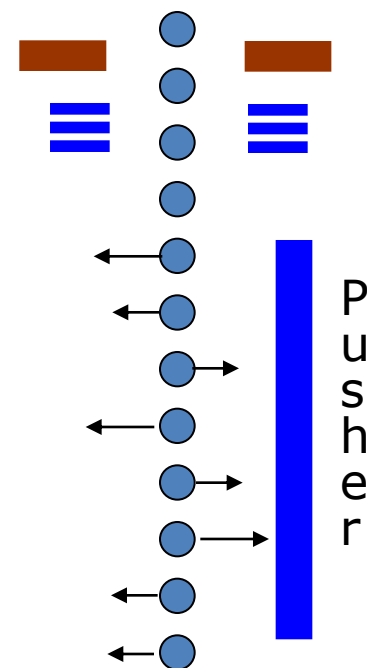
## Ideal Case:

perfectly straight line  
no orthogonal velocity component



## Spatial distribution:

some ions are closer to the plate than others



## Energy distribution:

small orthogonal velocity component



# Waters TOF Mass Spectrometers

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**Synapt  
G2-Si  
HDMS**

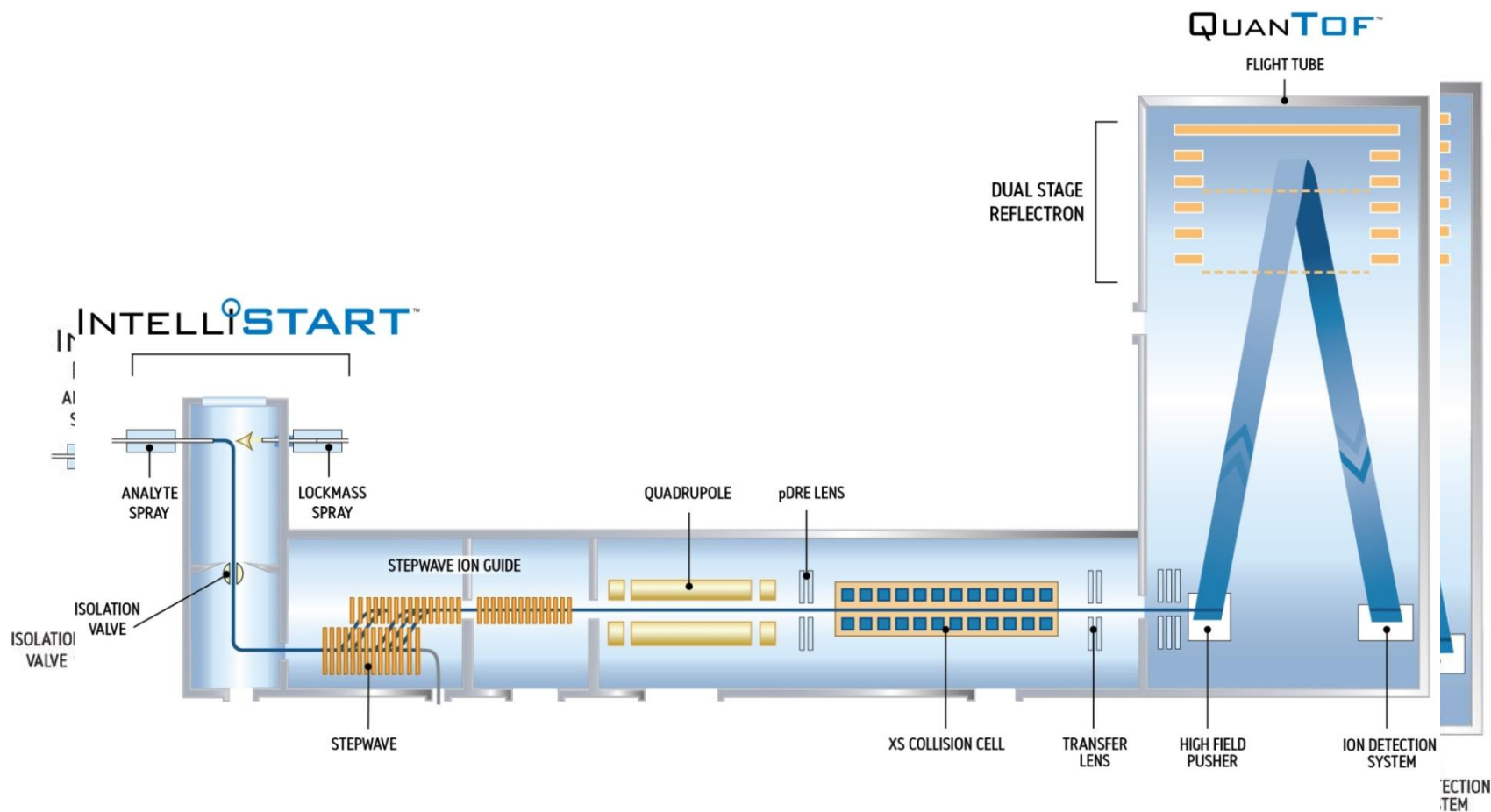


**Xevo  
G2-XS  
QToF**

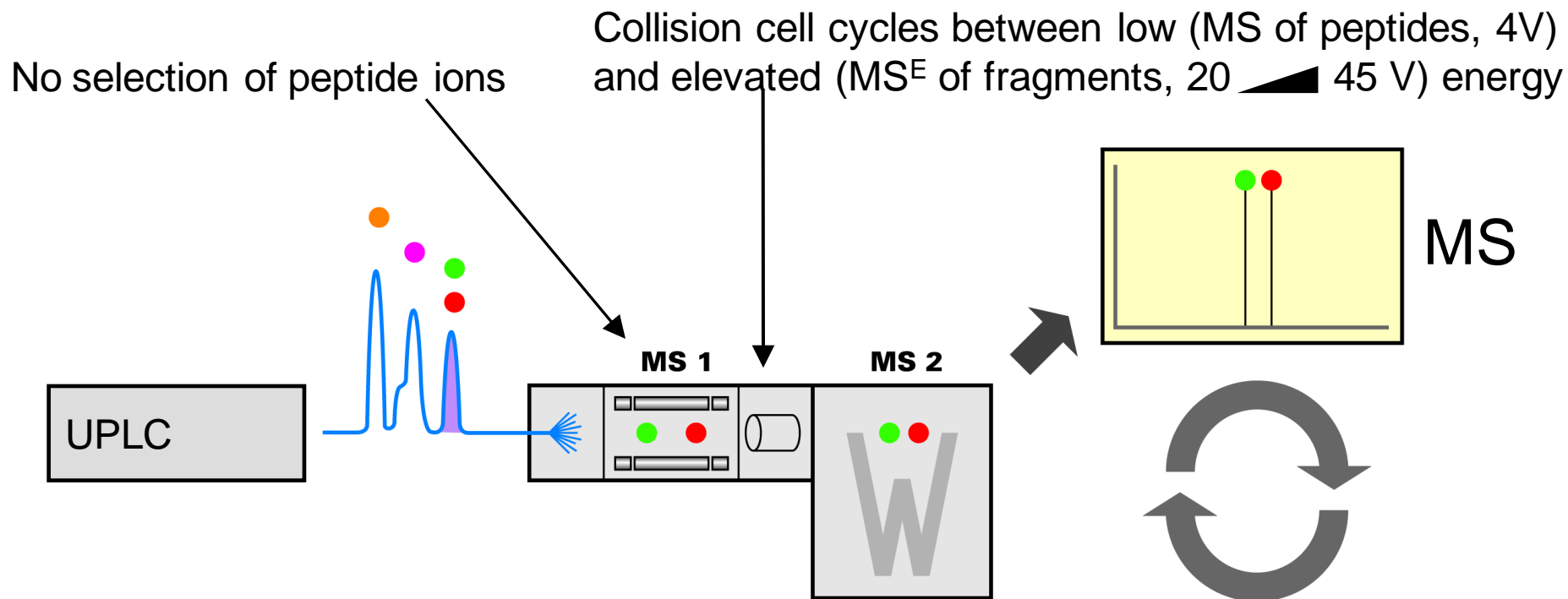


# Xevo G2-XS

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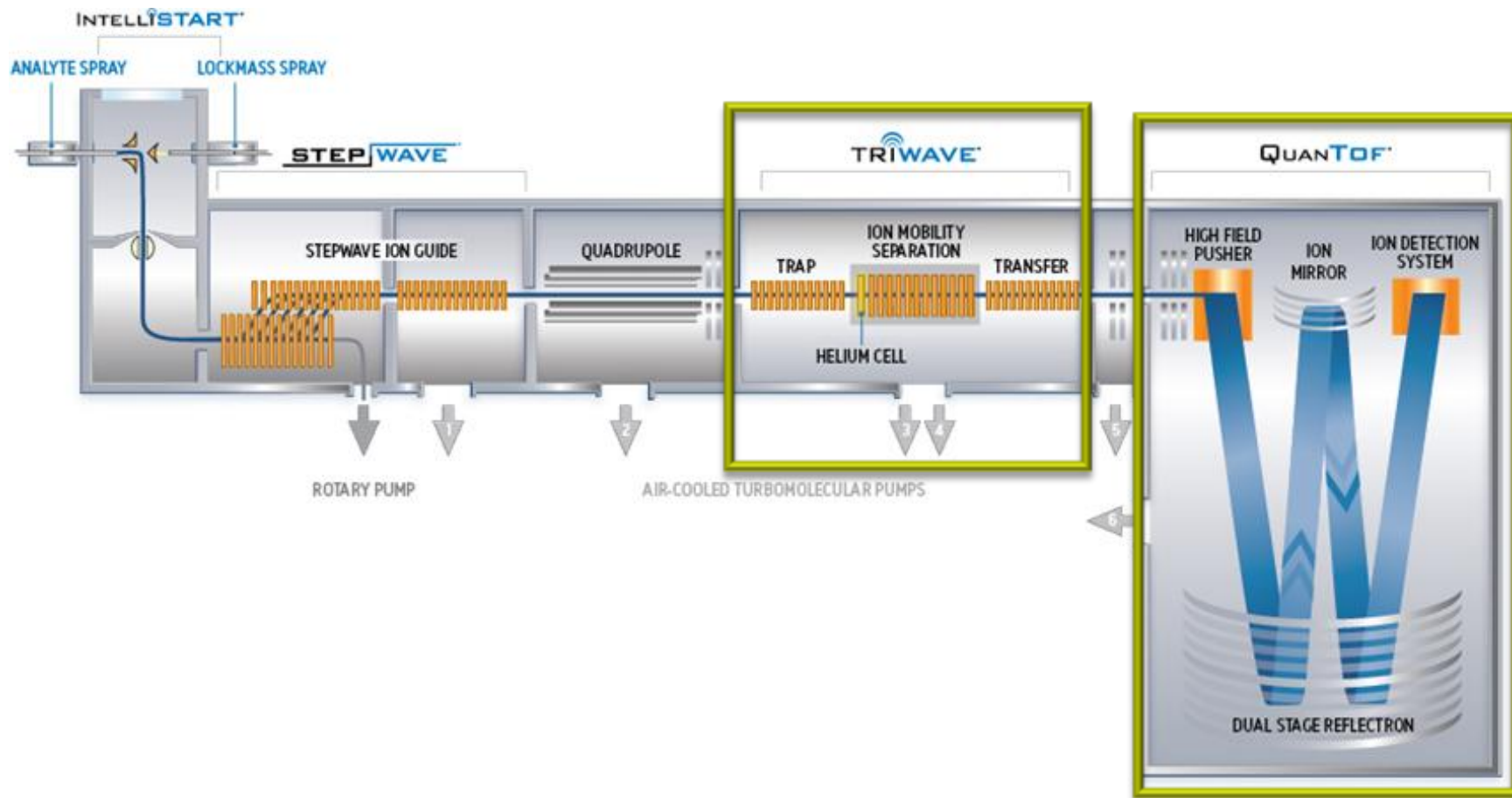


- Global analysis: acquire data on all the ions
- Maximize the LC/MS duty cycle
- Minimize bias/selection of ions (↑ Reproducibility)
- Obtain qualitative and quantitative information from the same analysis run



# SYNAPT G2-Si HDMS

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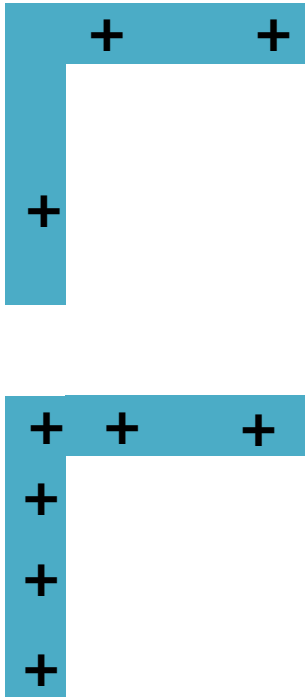


# **Ion mobility**



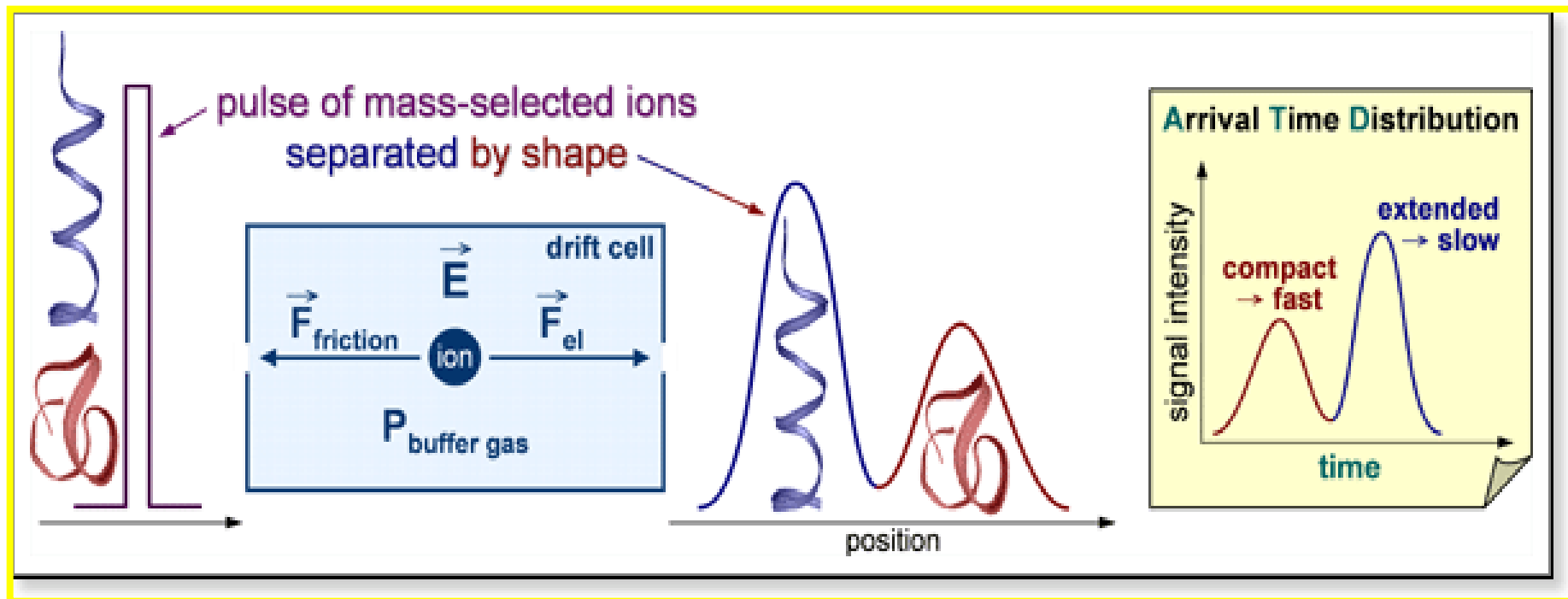
# Ion Mobility Separation (IMS)

The mobility of an ionised molecule is dependant on its...

		
Size	Shape	Charge



# “Classic” Ion Mobility Spectrometry



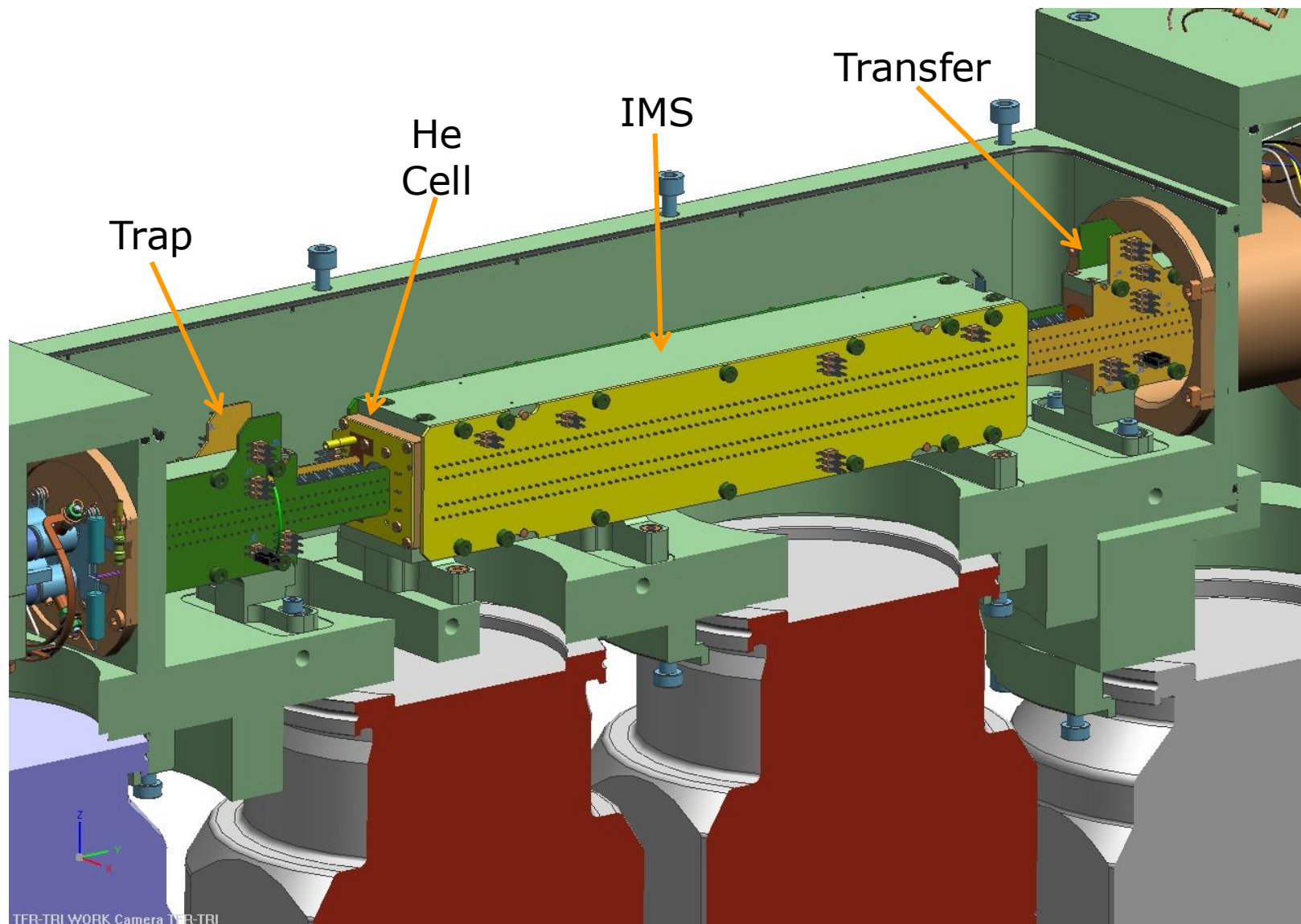
The Bowers Group Website [Ion Mobility Theory]:

[http://bowers.chem.ucsb.edu/theory\\_analysis/ion-mobility/index.shtml](http://bowers.chem.ucsb.edu/theory_analysis/ion-mobility/index.shtml)



# TriWave Device

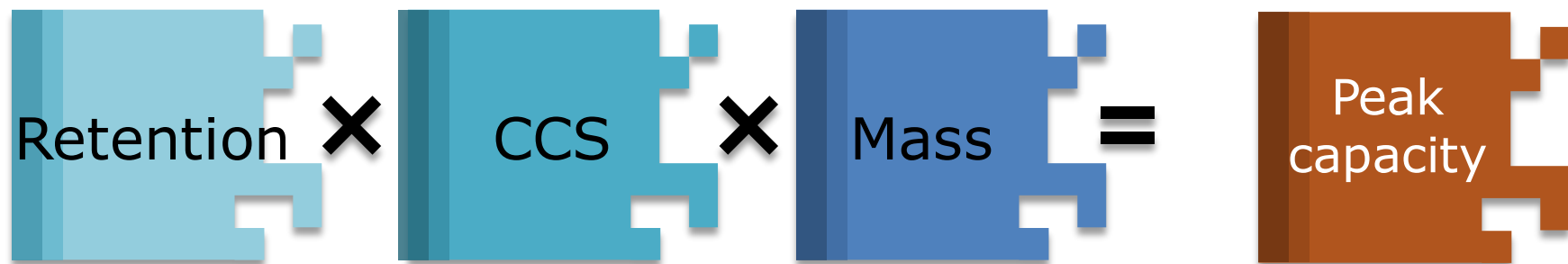
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Acquity  
UPLC®

T-WAVE™  
ION MOBILITY  
POWERED

QuanTof

UPLC/HDMS



# Mass Detection At Its Most Advanced

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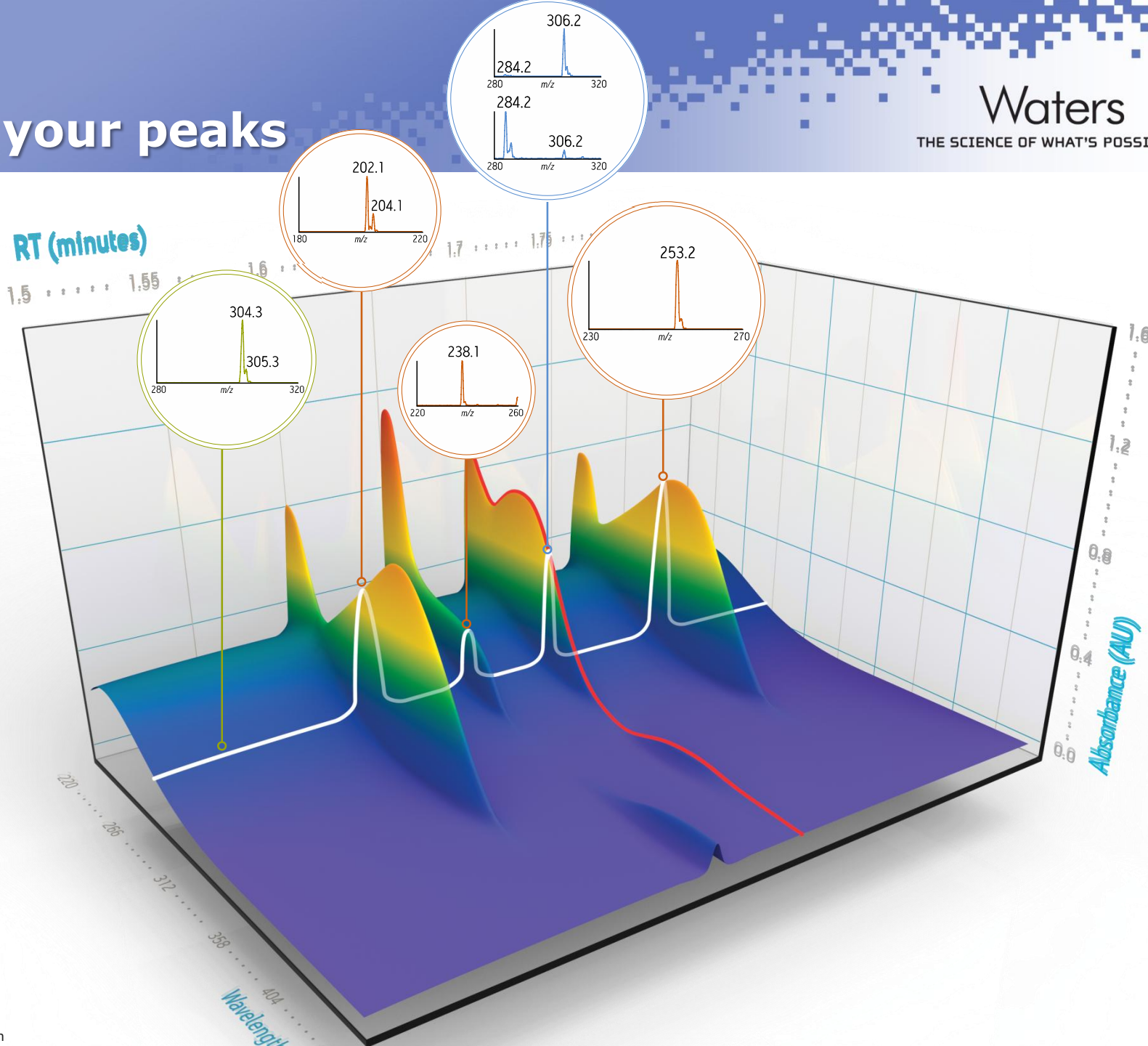
Acquity **QDa**





# Know your peaks

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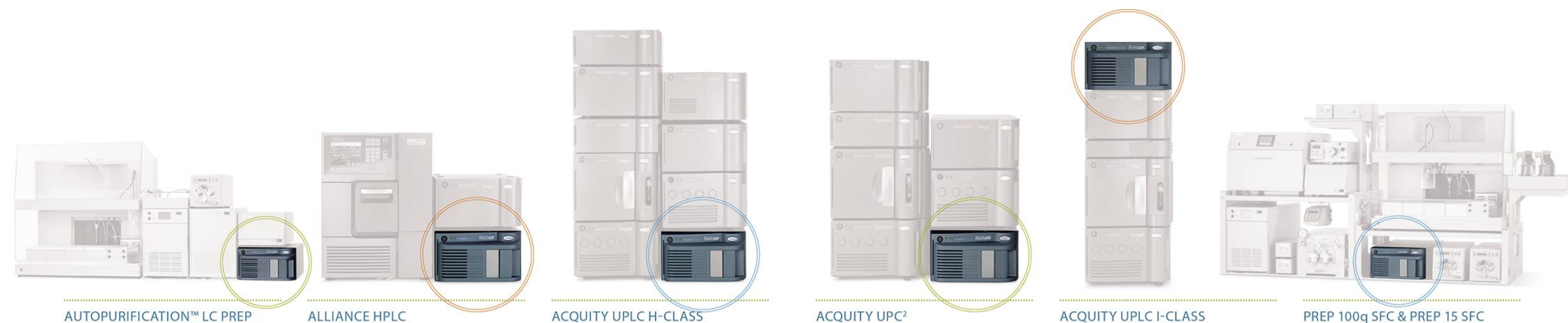


# Complementary & Compatible

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• 30-1250 DA • PRE-OPTIMIZED ES $\pm$  • 20 HZ FS • 100 HZ SIR • 4 ORDERS • INTUITIVE •

- Information-rich mass spectral data
- Targeted for small molecule applications
- Complementary to and compatible with PDA
  - UPLC, UPC<sup>2</sup>, purification
  - Optional ISM module to split and dilute
- Intuitive system for chromatographers





6.5 mins

22 mins

Waters

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**Thank You**

**Questions?**