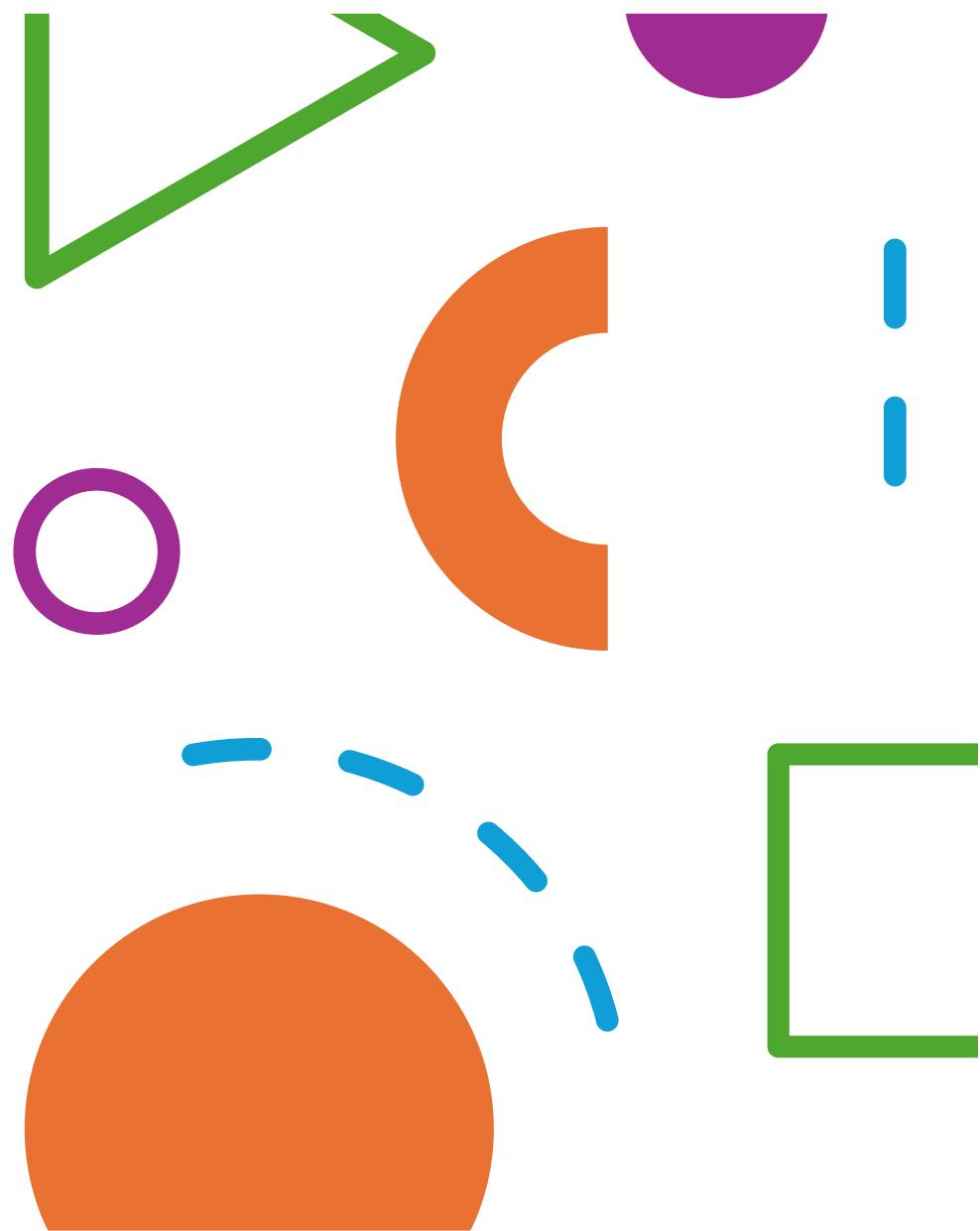


## How Does MS Work?

Ehsan Zangene  
5<sup>th</sup> session  
Time: 10-12  
Place: Biomedicum 1, kok.3



# **At the end of this session you should know:**

- Principles of mass spectrometry
  - Components of MS instruments
  - Tandem MS
  - Data acquisition techniques
  - DDA/DIA
  - TMT labelling

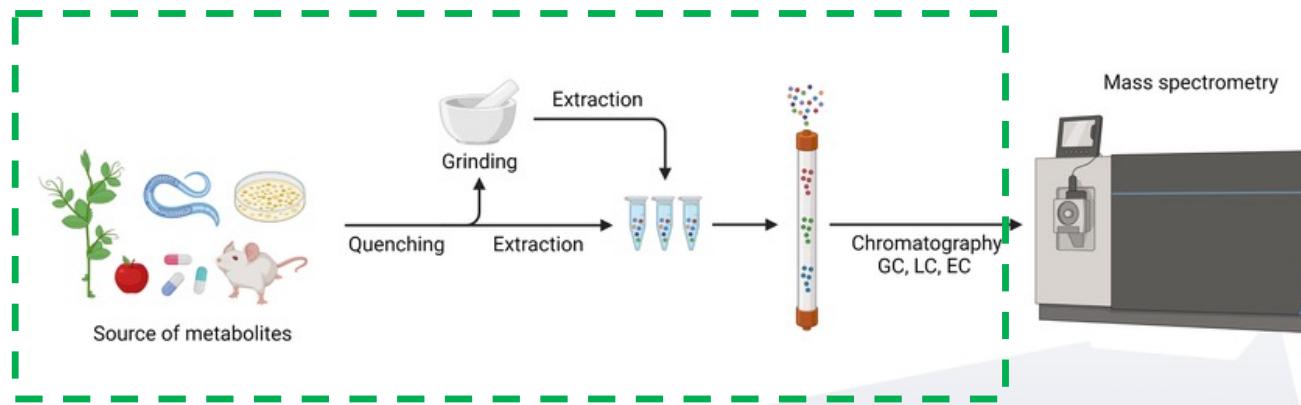


Individual assignment

10 min



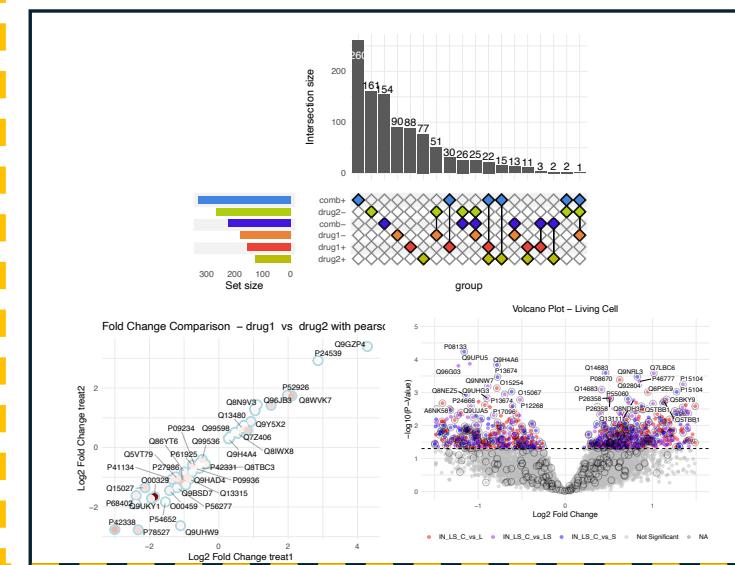
# workflow



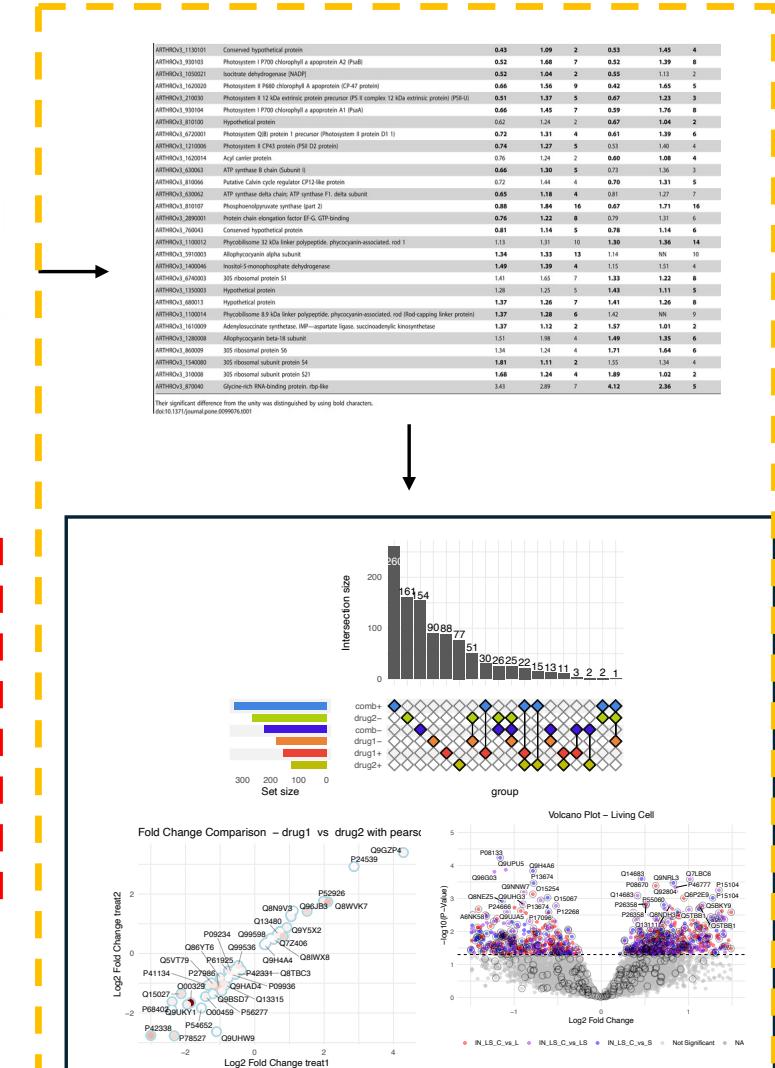
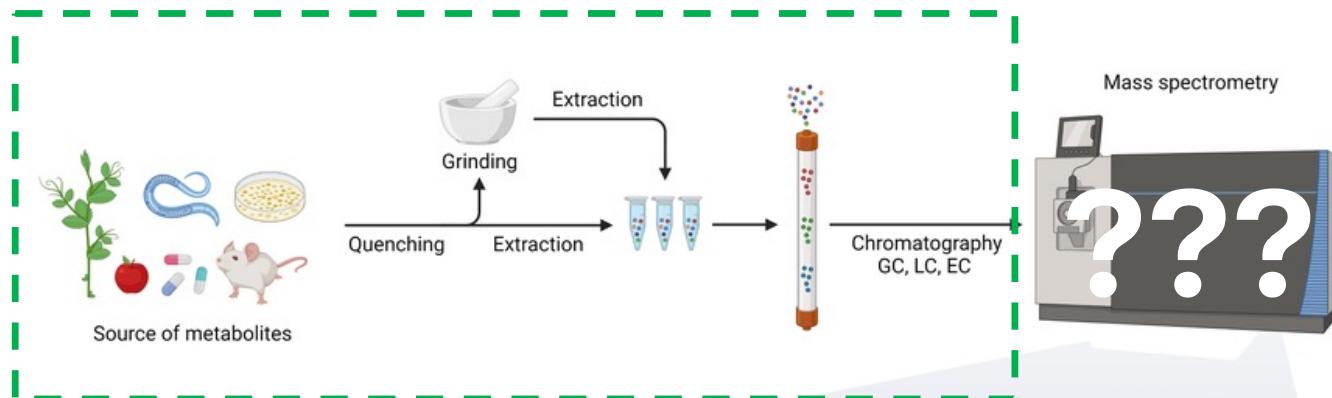
?

<https://pel.caltech.edu/approach>

ATHOMA_1130101	Conserved hypothetical protein	0.43	1.09	2	0.53	1.45	4
ATHOMA_1130102	Phosphotyrosyl phosphatase A2 (PahI)	0.52	1.69	7	0.32	1.39	9
ATHOMA_1130201	Iodothyrodeiodinase (NADH)	0.52	1.04	2	0.55	1.11	2
ATHOMA_1162030	Phosphotyrosyl A2 phosphotyrosyl A2 (T4T protein)	0.66	1.56	9	0.42	1.65	5
ATHOMA_2103001	Proteasome I 12 kDa extrinsic protein precursor (PS-I complex) 12 kDa extrinsic protein (PS-II)	0.51	1.37	5	0.67	1.23	1
ATHOMA_1130104	Phosphotyrosyl A2 phosphotyrosyl A1 (Puk)	0.66	1.45	7	0.39	1.76	8
ATHOMA_1130105	Hypothetical protein	0.62	1.24	2	0.67	1.04	2
ATHOMA_0720001	Phosphotyrosyl Q8B1 protein I precursor (Phosphotyrosyl protein D1)	0.72	1.31	4	0.61	1.39	1
ATHOMA_1130106	Phosphotyrosyl Q8C3 protein (PS-II protein)	0.72	1.31	4	0.61	1.39	4
ATHOMA_1130104	Arg/Cys-rich protein (PS-II protein)	0.16	1.21	4	0.60	1.08	4
ATHOMA_0300003	ATP synthase B chain (Subunit II)	0.66	1.30	5	0.73	1.30	5
ATHOMA_1130106	Putative Calvin cycle regulator CP2 (ATP synthase C chain)	0.72	1.44	4	0.70	1.31	5
ATHOMA_0300202	ATP synthase delta chain, ATP synthase F1-like delta subunit	0.65	1.18	4	0.81	1.27	7
ATHOMA_1130107	Phosphotyrosylphosphate synthase (part 2)	0.88	1.84	16	0.67	1.71	16
ATHOMA_2890001	Protein chain elongation factor G-1, GTP-binding	0.76	1.22	8	0.79	1.31	6
ATHOMA_760041	Conserved hypothetical protein	0.81	1.14	5	0.78	1.14	6
ATHOMA_1130102	Phosphotyrosyl D2-like linker polypeptide, phosphocyanin-associated, rod 1	1.13	1.31	10	1.30	1.36	14
ATHOMA_1130103	Allophycocyanin alpha subunit	1.13	1.31	10	1.30	1.36	10
ATHOMA_1130104	Phosphotyrosyl dipeptidyl phosphotriester hydrolase	1.49	1.59	15	1.15	1.55	15
ATHOMA_0400003	3D-protein scaffold protein 51	1.41	1.65	7	1.33	1.32	5
ATHOMA_1130051	Hypothetical protein	1.28	1.25	5	1.43	1.11	5
ATHOMA_0600103	Hypothetical protein	1.37	1.26	7	1.41	1.26	8
ATHOMA_1130014	Physiological 8.1 kDa linker polypeptide, physocyanin-associated, rod 1 (lidding linker protein)	1.37	1.28	6	1.42	0.99	5
ATHOMA_1610009	Adenylosuccinate synthetase, AMP--aspartate ligase, succinoadenyl kinase synthetase	1.37	1.12	2	1.57	1.01	2
ATHOMA_1230008	Allophycocyanin beta-18 subunit	1.51	1.98	4	1.49	1.33	6
ATHOMA_3600009	3D-protein scaffold protein 56	1.51	1.24	4	1.71	1.64	6
ATHOMA_1130001	Phosphotyrosyl D2-like linker polypeptide, phosphocyanin-associated, rod 2	1.51	1.25	10	1.50	1.35	10
ATHOMA_3100008	3D-protein scaffold protein 53	1.66	1.24	18	1.09	1.02	2
ATHOMA_3700001	Growth arrest-specific 5 homolog, pro-apoptotic, mRNA	1.61	1.93	2	1.33	1.36	5



# workflow

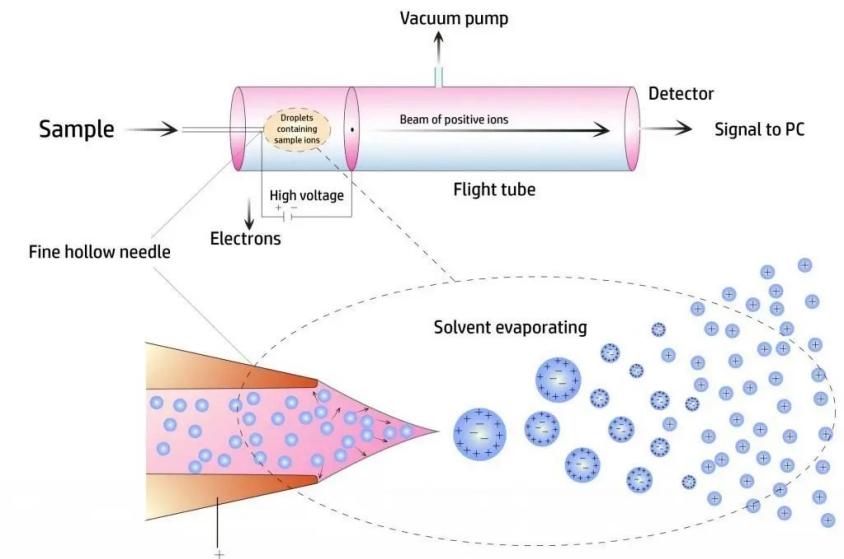


<https://pel.caltech.edu/approach>

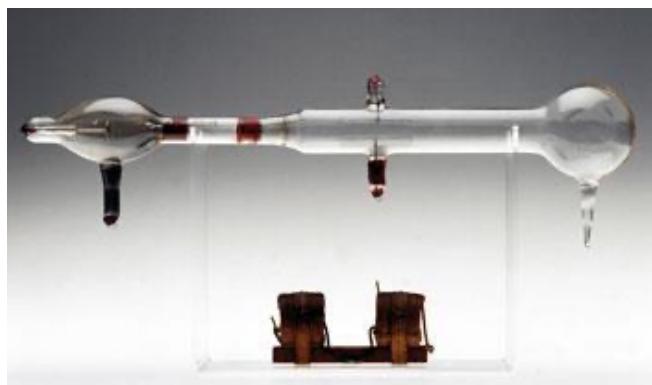
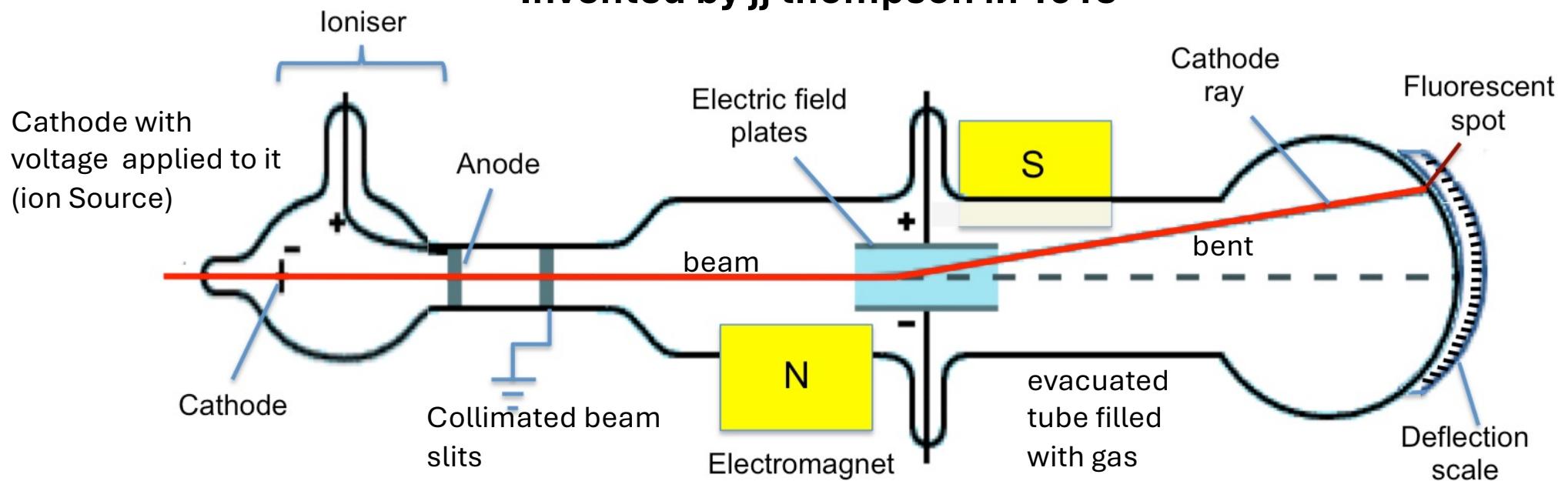
# Principles of mass spectrometry

- generating charged particles (ions) to generate ion beam
- ion beam manipulated under high vacuum
- measure ions mass to charge ratio
  - Parent ions
  - daughter ions (fragmented)

mass spectrometers  
do not measure mass 



## Invented by jj thompson in 1913



Two mass filters

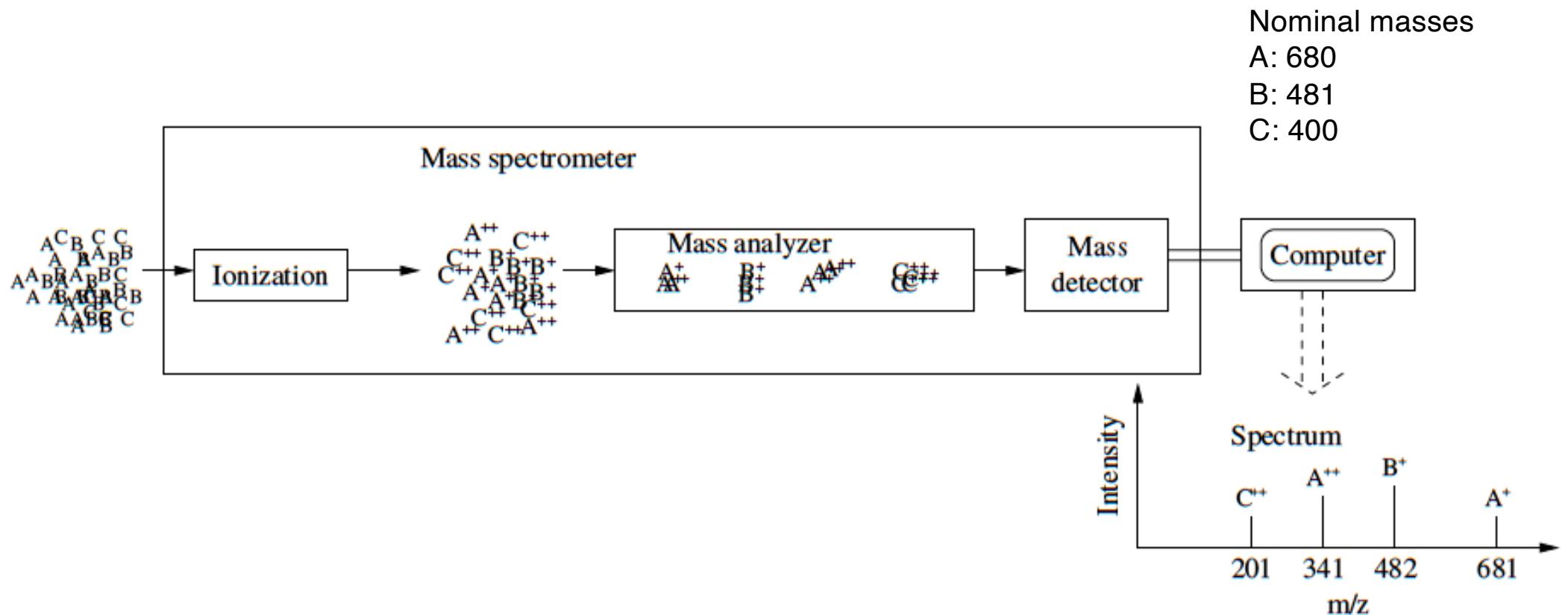
- magnetic coils -> magnetic field
- electrical diffraction plates -> electrical field

detector -> fluorescent coating

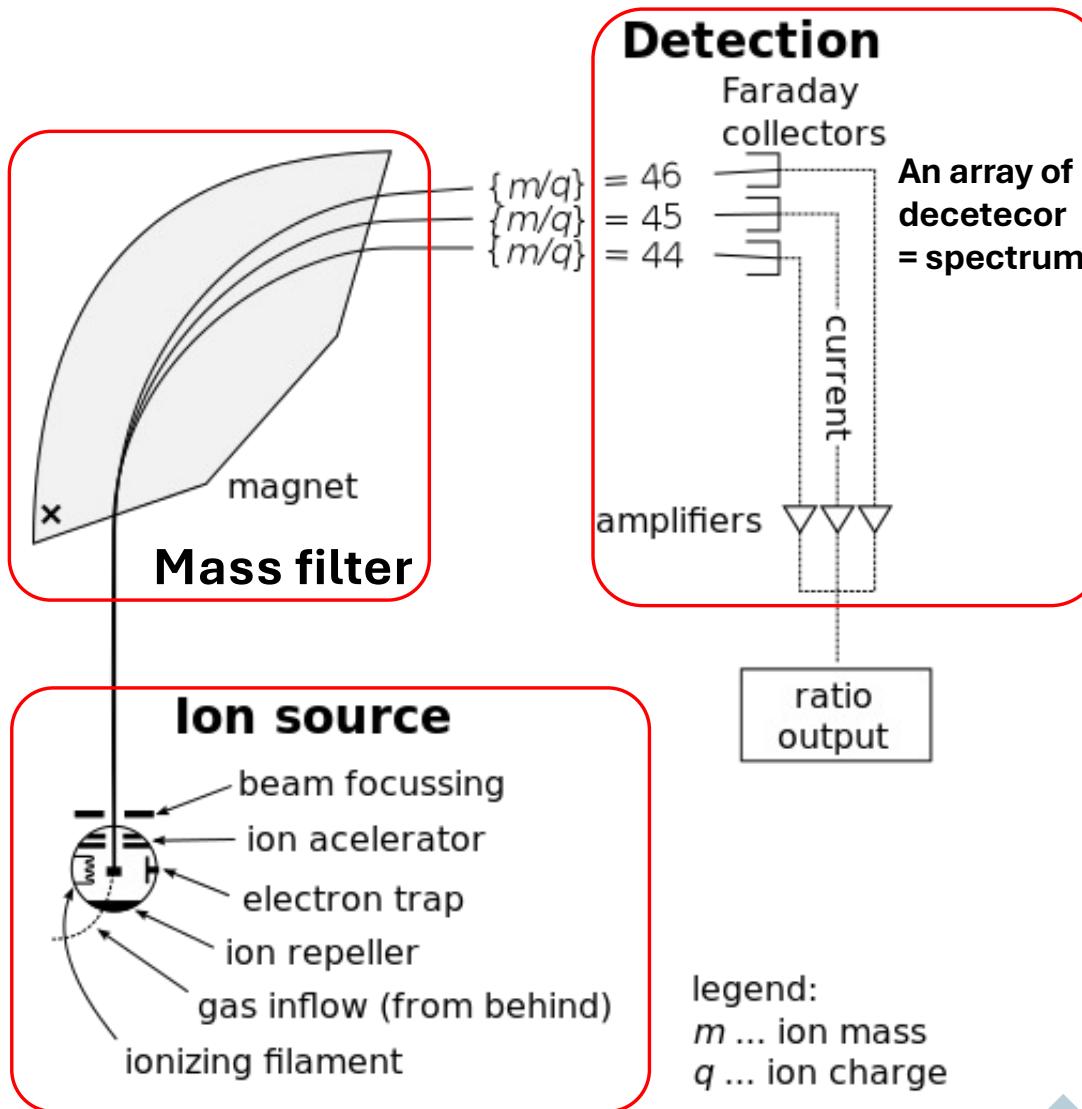
it will fluoresce when charged particle beam hits

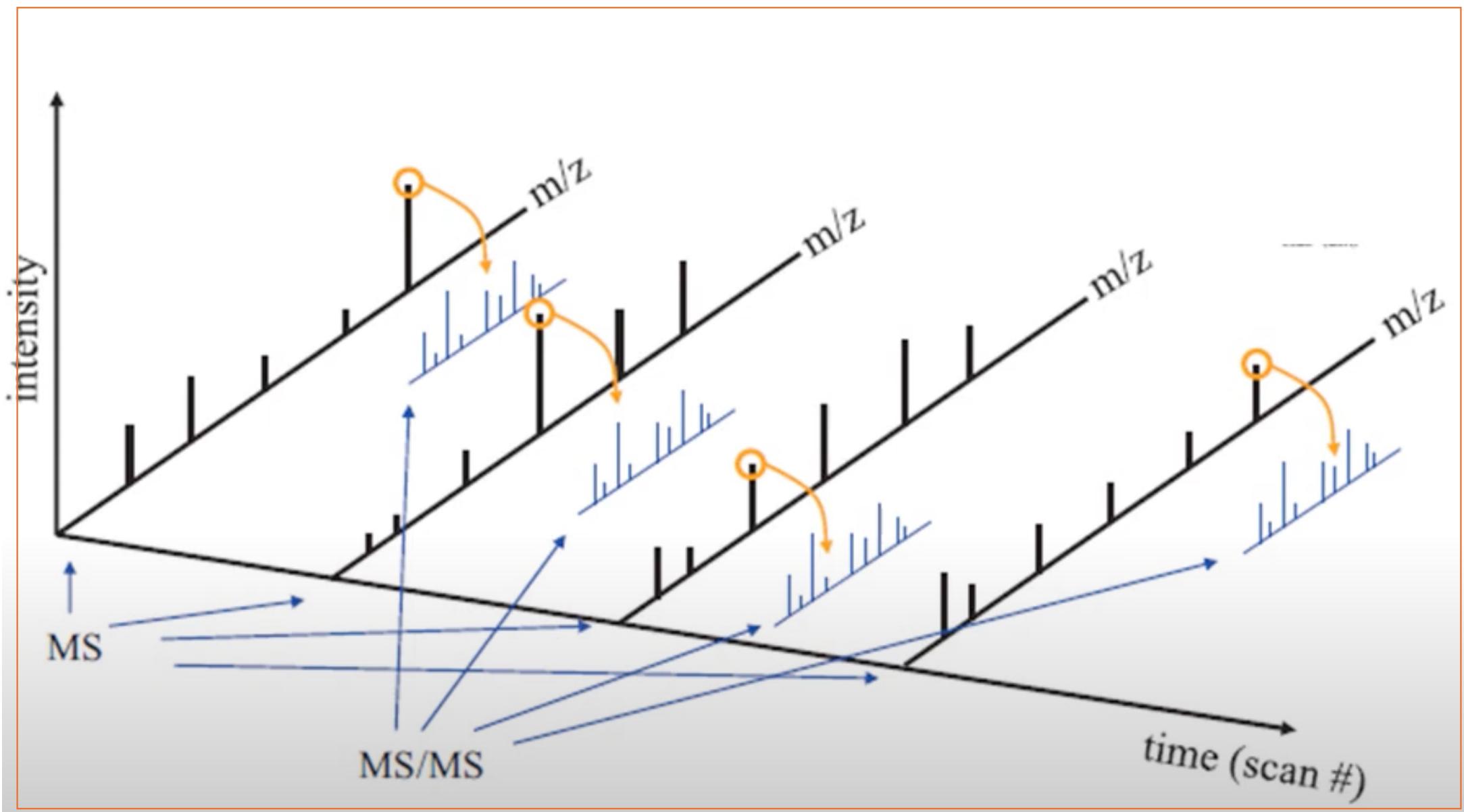
He was able to separate different Neon isotope

<https://monomole.com/mass-spectrometry-thomson-cathode-ray/>



- A:  $(680+1)/1=681$  and  $(680+2)/2=341$
  - B: 482
  - C: 201



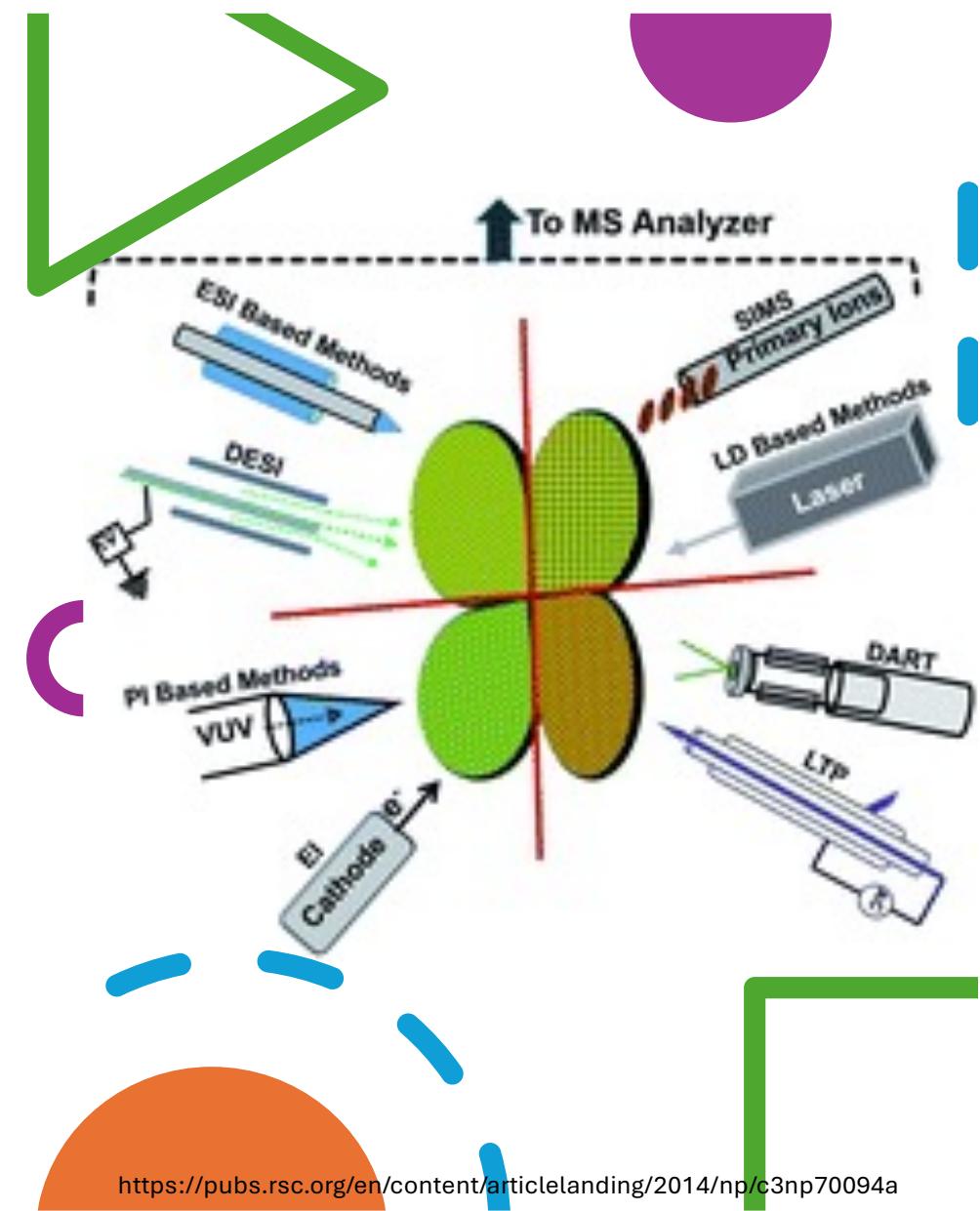


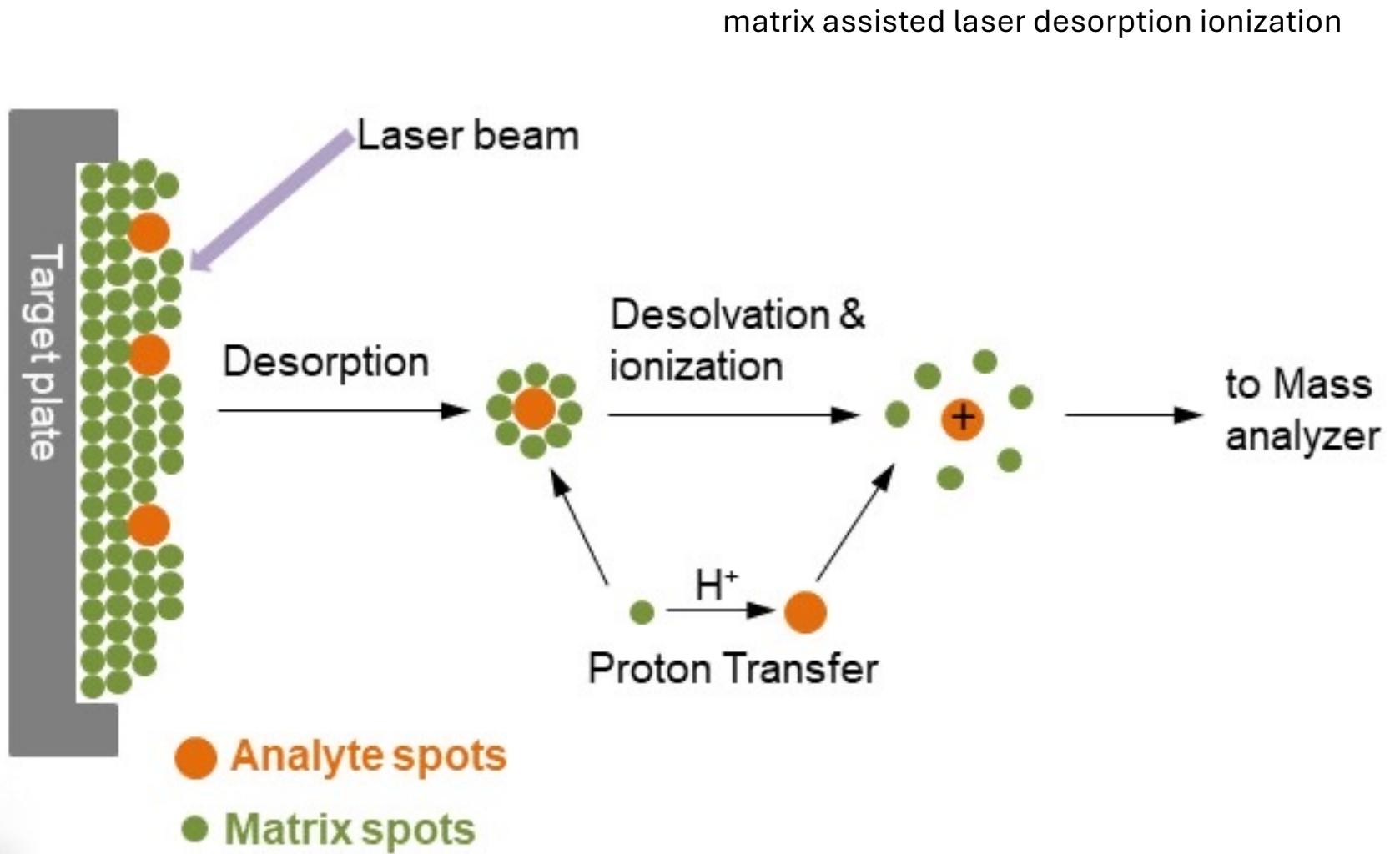
# Ionization sources

Electrospray

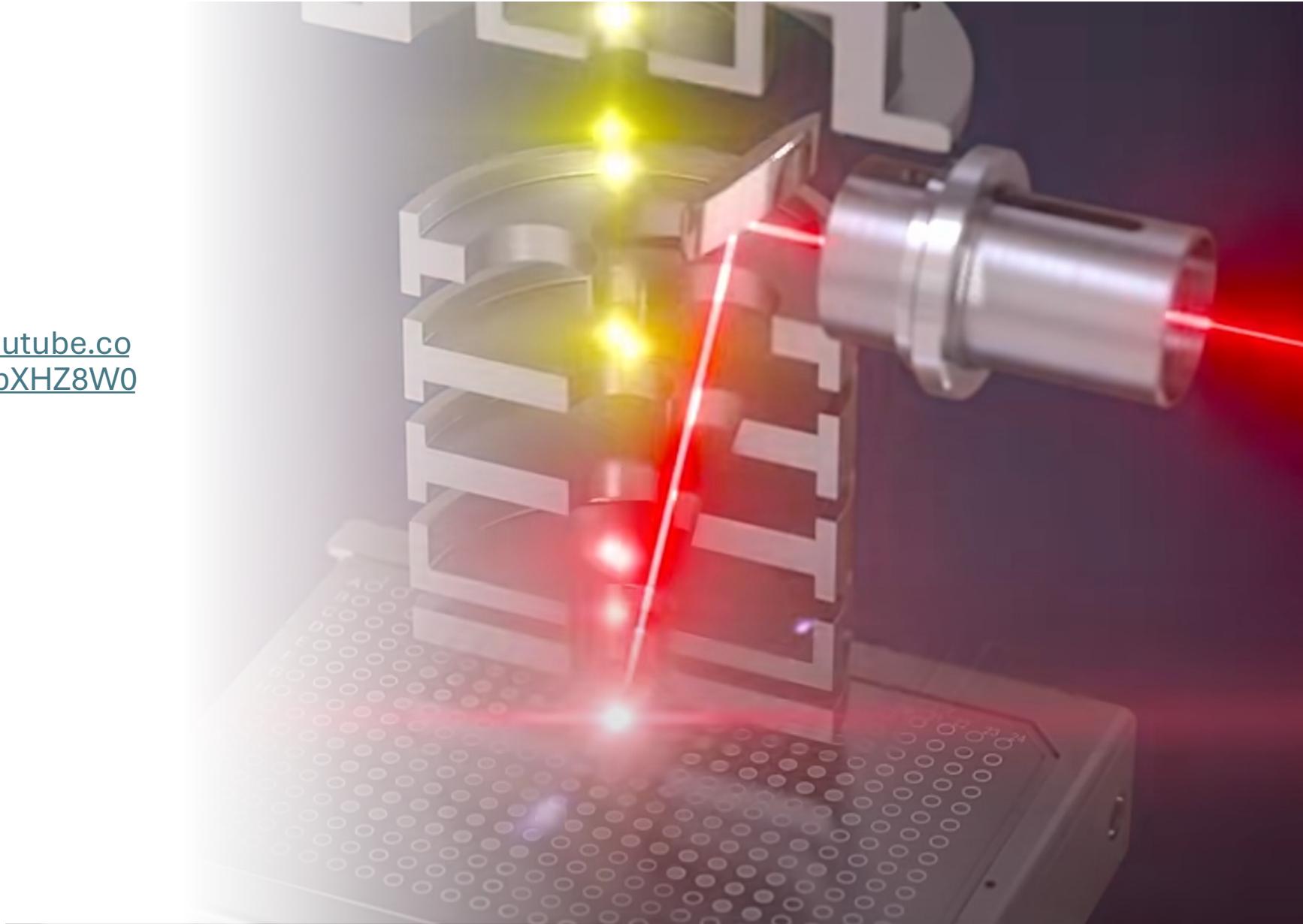
MALDI

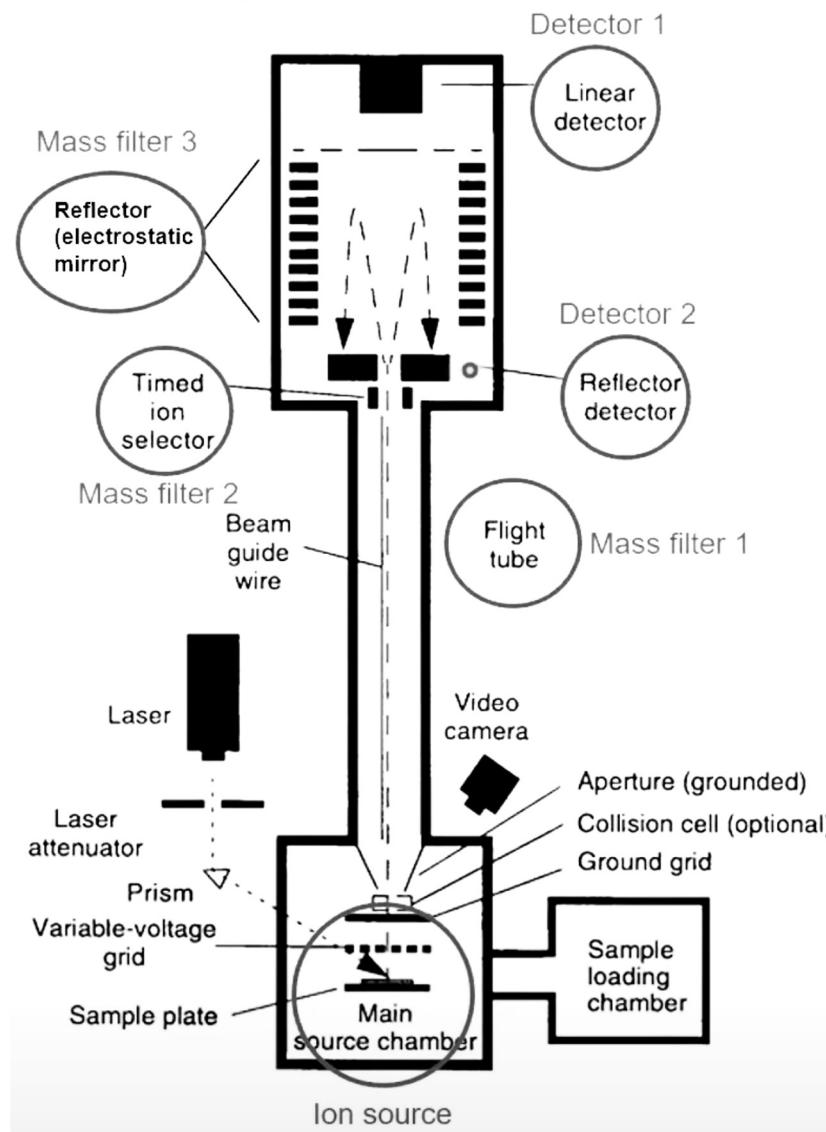
Other  
methods

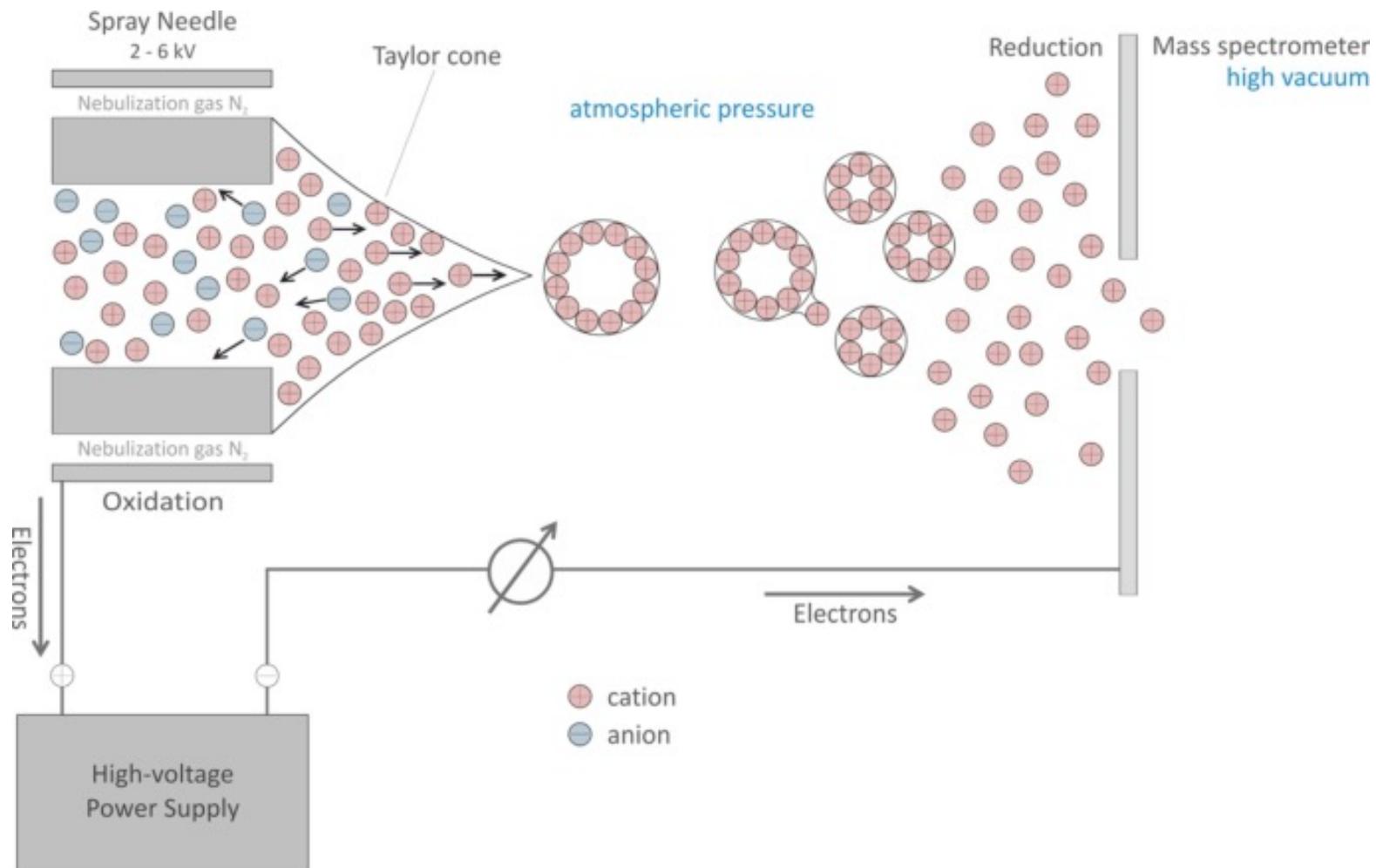




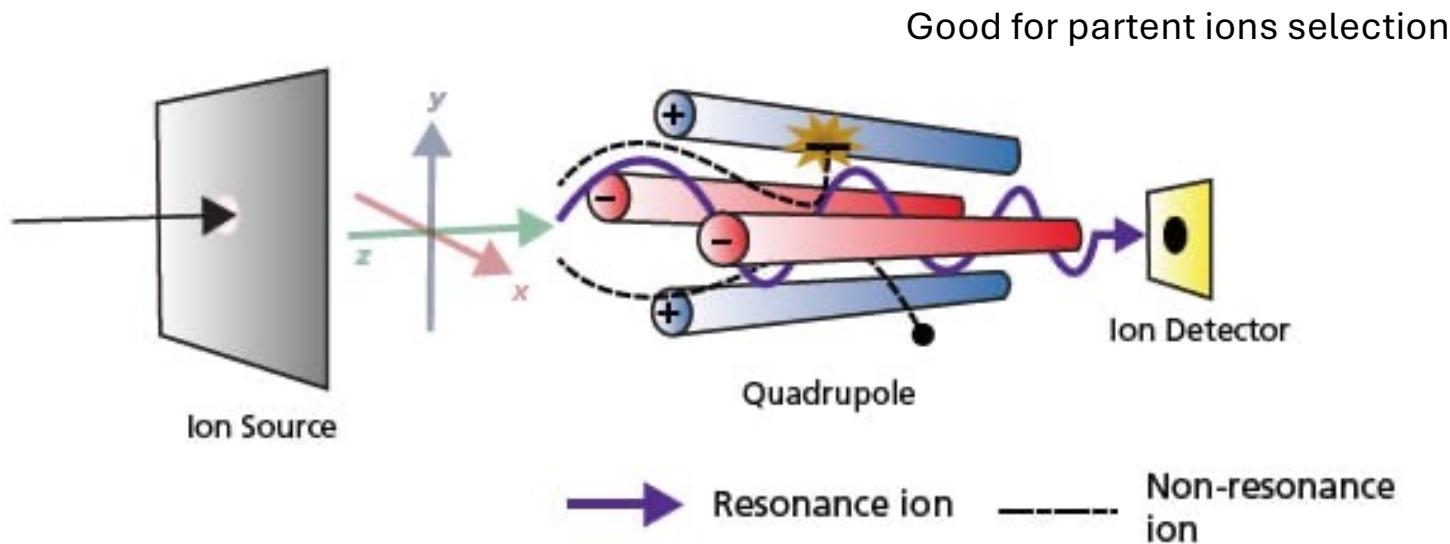
- <https://www.youtube.com/watch?v=0jeFpXHZ8W0>



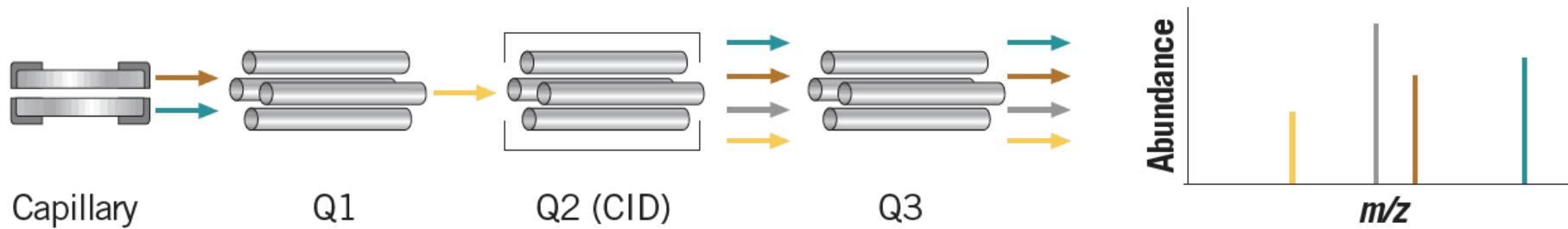




<https://www.youtube.com/watch?v=9AWBAI-Owzk>



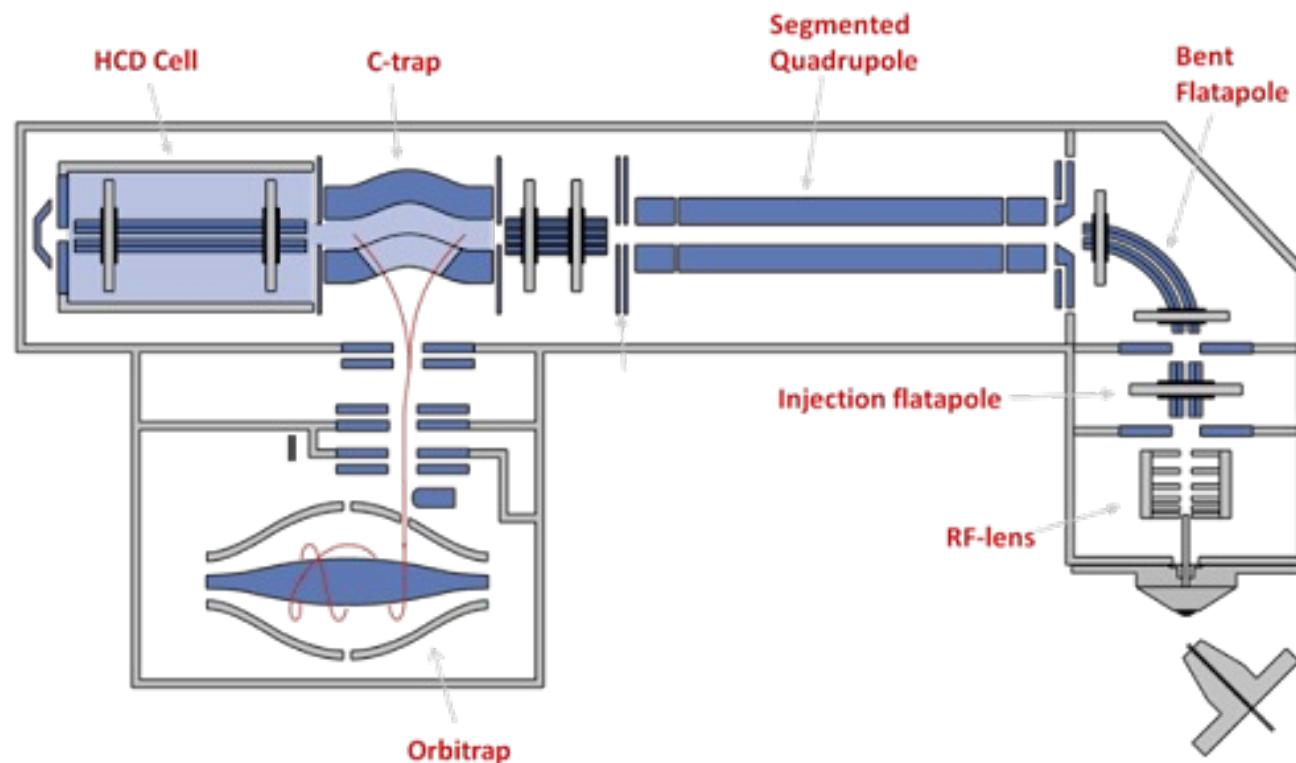
## MS/MS IN A TRIPLE-QUADRUPOLE MASS SPECTROMETER



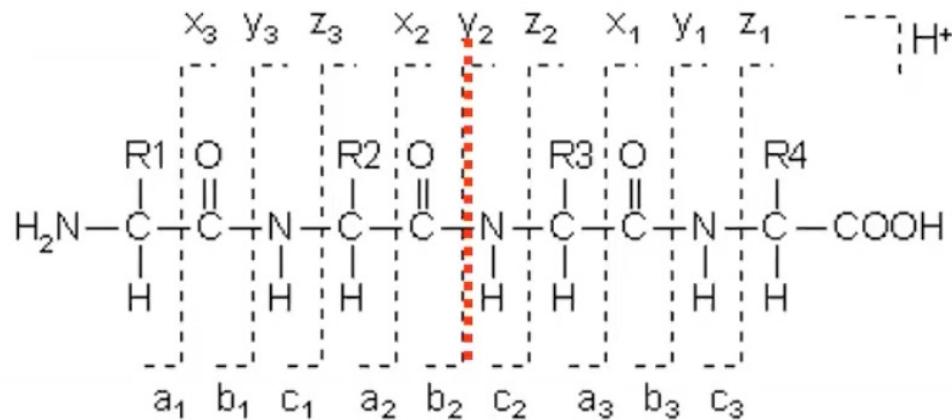
Lubrizol Life Science



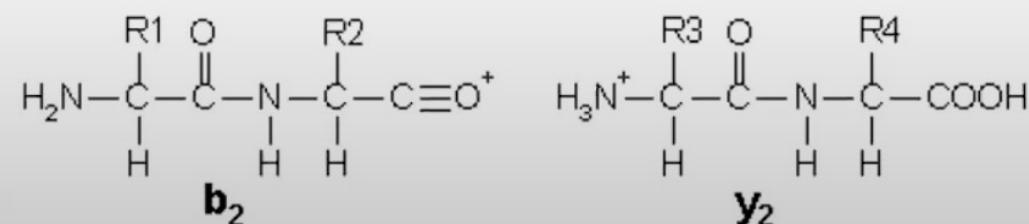
<https://www.youtube.com/watch?v=W-DRL-V2Rkg>



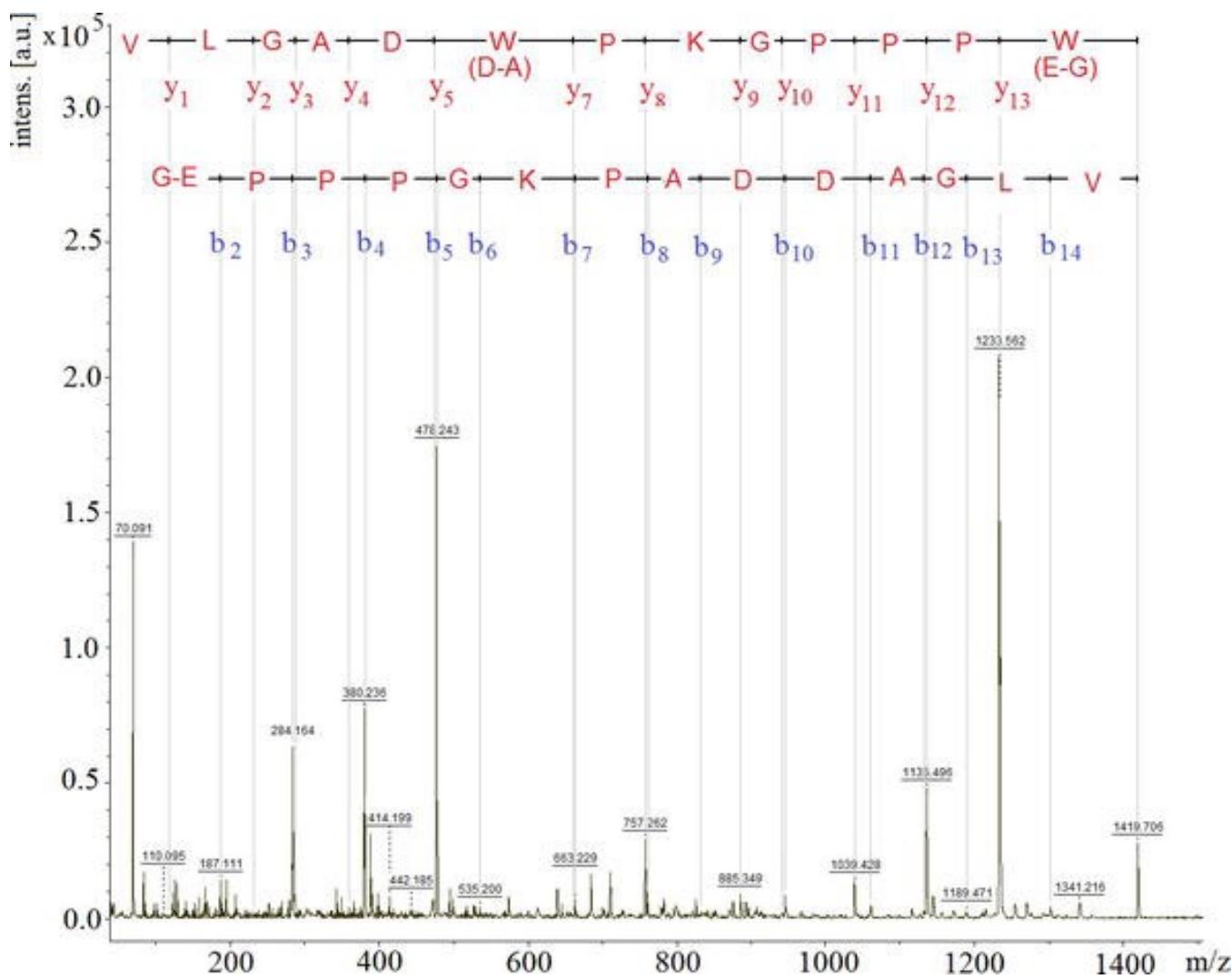
<https://www.youtube.com/watch?v=RsFsaCkVqxM>



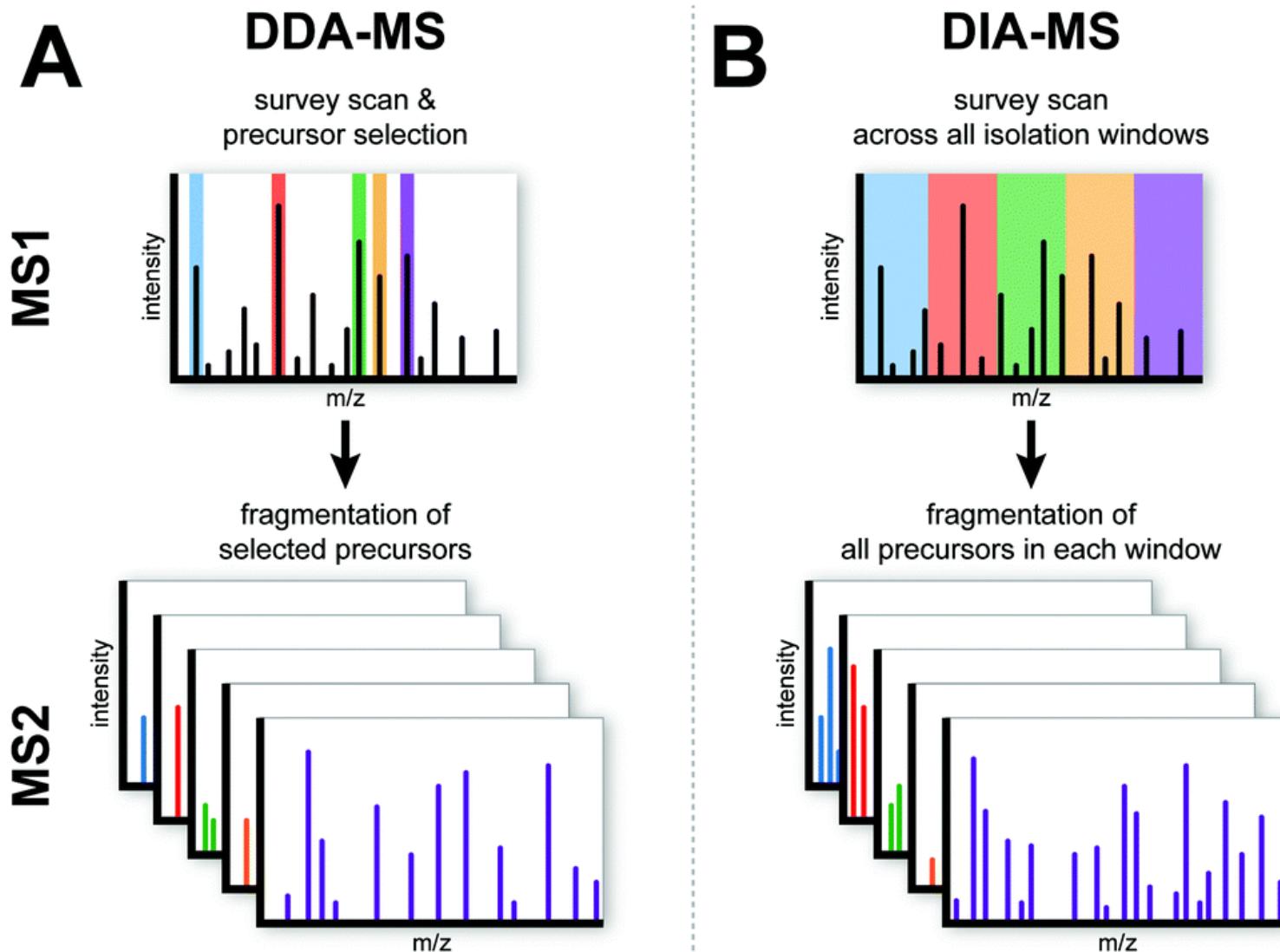
**parent ion**



**daughter  
ions**



## 1. Data Acquisition technique



## 1. Data-Dependent Acquisition (DDA)

- 📌 **Most common** in discovery proteomics
  - ◆ MS<sup>1</sup> scans all ions
  - ◆ Selects the **top N most intense ions**
  - ◆ Fragments them (MS<sup>2</sup>) → identifies peptides
-  Pros: good ID quality
-  Cons: may miss low-abundance peptides (biased)

## 2. Data-Independent Acquisition (DIA)

- 📌 Newer and increasingly popular
  - ◆ MS scans **all ions in defined m/z windows** (not just top ones)
  - ◆ Fragments **everything** in those windows
  - ◆ Generates complex MS<sup>2</sup> spectra, analyzed with advanced software
- ✅ Pros: **more comprehensive, less biased**  
❌ Cons: complex data, needs good spectral libraries

Group assignment

15 min



PPT file -> <https://github.com/esnzgn/MSPROT>



Designed by me

