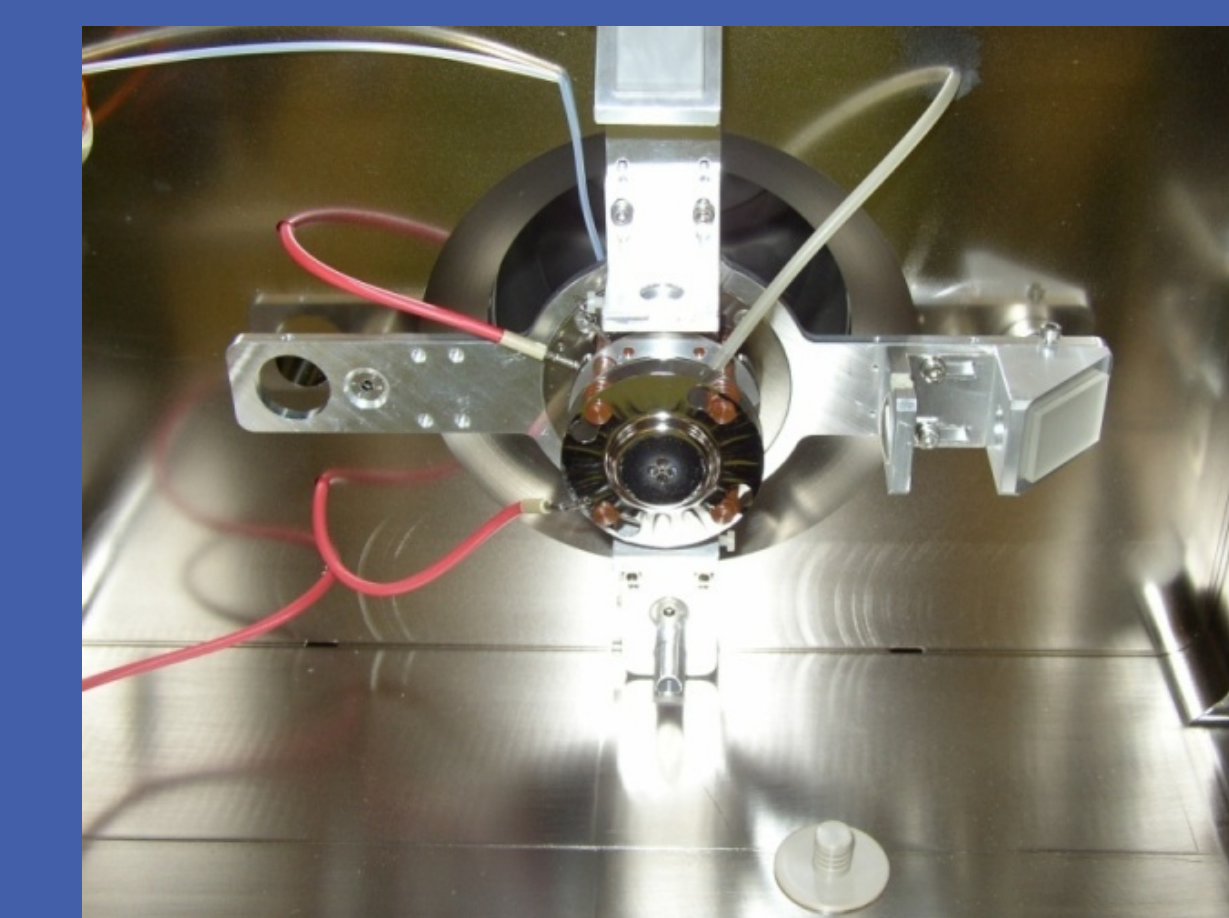


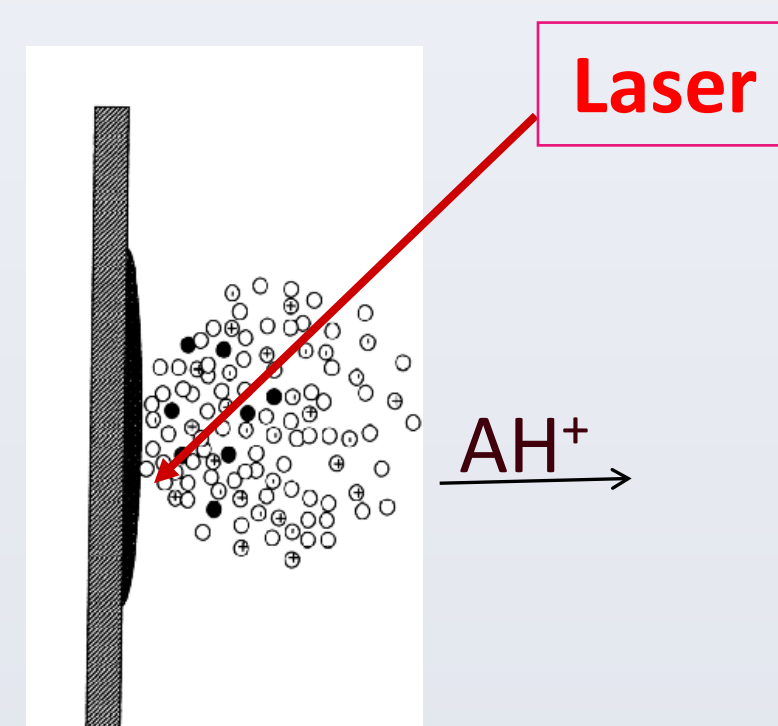
# Matrix Assisted Laser Desorption Ionization – Time of Flight

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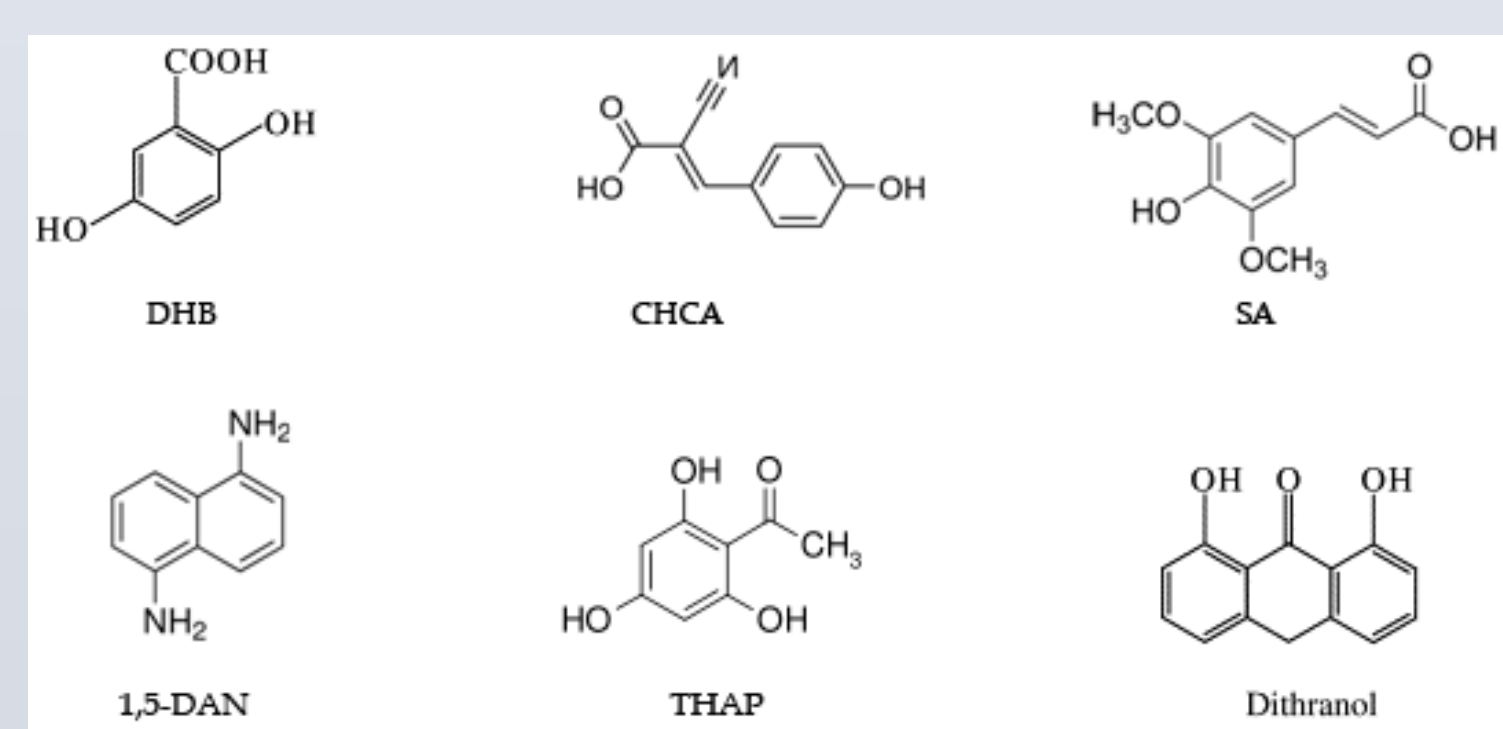


## Matrix Assisted Laser Desorption Ionization (MALDI)



1. Either sample (A) is mixed with excess matrix (M) and dried on a MALDI plate or solutions of M and A are placed on the plate sequentially.
2. Laser flash ionizes matrix molecules.
3. Sample molecules are ionized by proton transfer from matrix:  
 $MH^+ + A \rightarrow M + AH^+$

### Common MALDI matrices



### MALDI Targets



2,5-dihydroxy benzoic acid crystallized on stainless steel target



2,5-dihydroxy benzoic acid crystallized on AnchorChip target

During solvent evaporation, sample shrinks onto the hydrophilic anchors (200-800 μm) in hydrophobic surroundings.  
Enhance sensitivity by a factor of 10-100.

## Time of Flight (TOF)

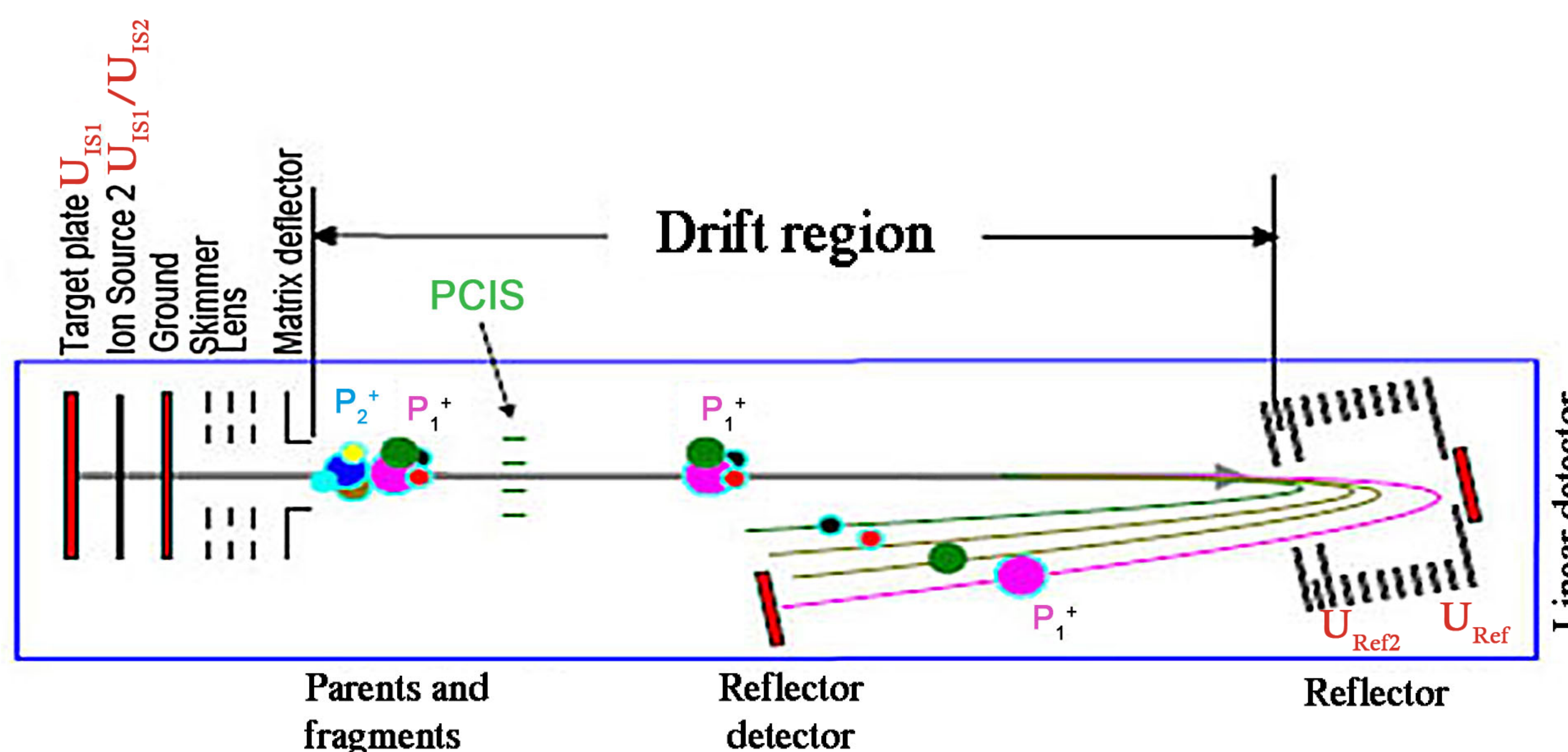
$$\frac{m}{z} = \frac{2t^2U}{L^2}$$

$t$  = drift time,  $L$  = drift length  
 $m$  = mass,  
 $U$  = acceleration voltage  
 $z$  = number of charges on ion

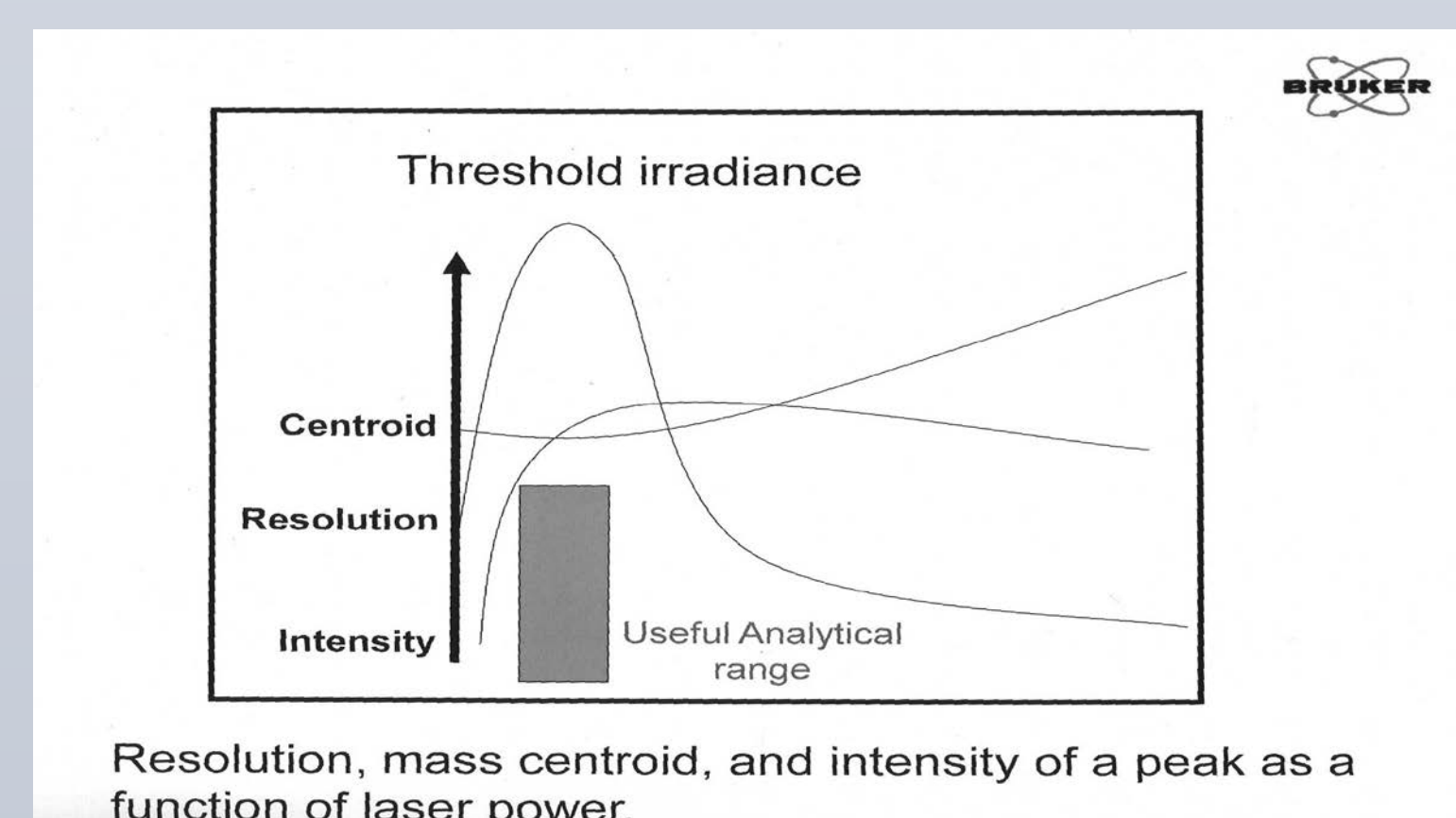
If  $U = 20$  KV,  $L = 1.8$ m,  $m = 1000$  Da,  $z = +1$

$$t = \frac{1.8m}{\sqrt{2 \times 20000V}} \sqrt{\frac{1000\text{Da} \times 1.660539 \times 10^{-27} \text{ kg Da}^{-1}}{1.602 \times 10^{-19} \text{ C}}} = 2.9 \times 10^{-5} \text{ s}$$

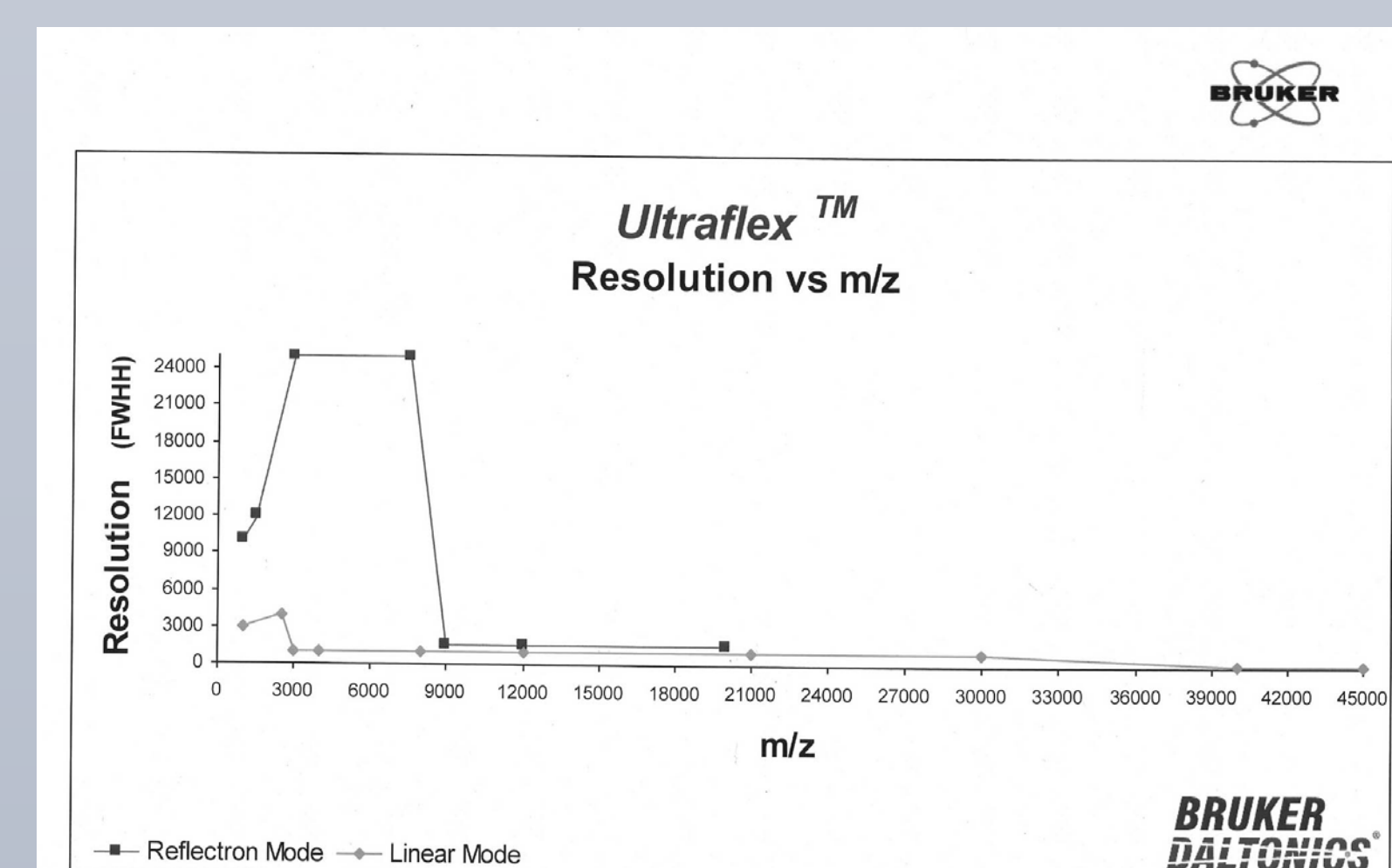
## Instrument Schematics and tuning



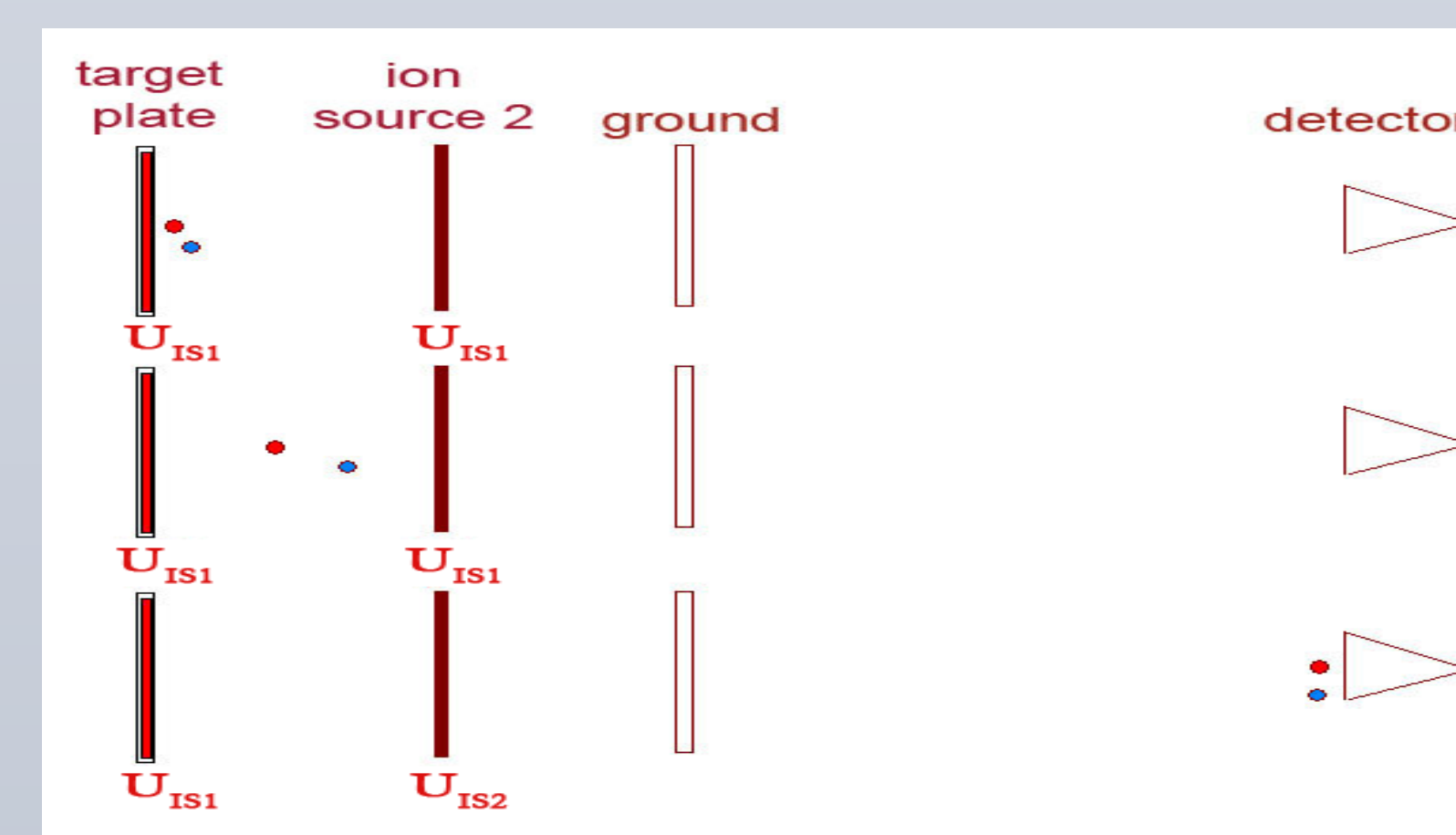
$U_{IS1}$  (~20-25 KV) &  $U_{IS2}$  (~85-95% of  $U_{IS1}$ ), acceleration voltages applied on Ion Source 1 and Ion Source 2 plates  
 $U_{ref}$  (~21-26.5 KV) &  $U_{ref2}$  (~50-60% of  $U_{ref}$ ), reflector and reflector 2 voltages  
PCIS, Precursor Ion Selector;  $P_1^+$  and  $P_2^+$ , precursor ions  
**Flight tube length:** linear, 182cm; reflectron, 320 cm  
**Laser:** MNL 100 N<sub>2</sub> laser, LTB Lasertechnik Berlin, 337nm, 150μJ/3ns, beam dimension, 3 x 4 mm, focused spot size, ~0.1 mm



### Laser Power



### Linear or reflectron analyzer



### Pulsed Ion Extraction (PIE) delay

### Two Stage Ion Reflector

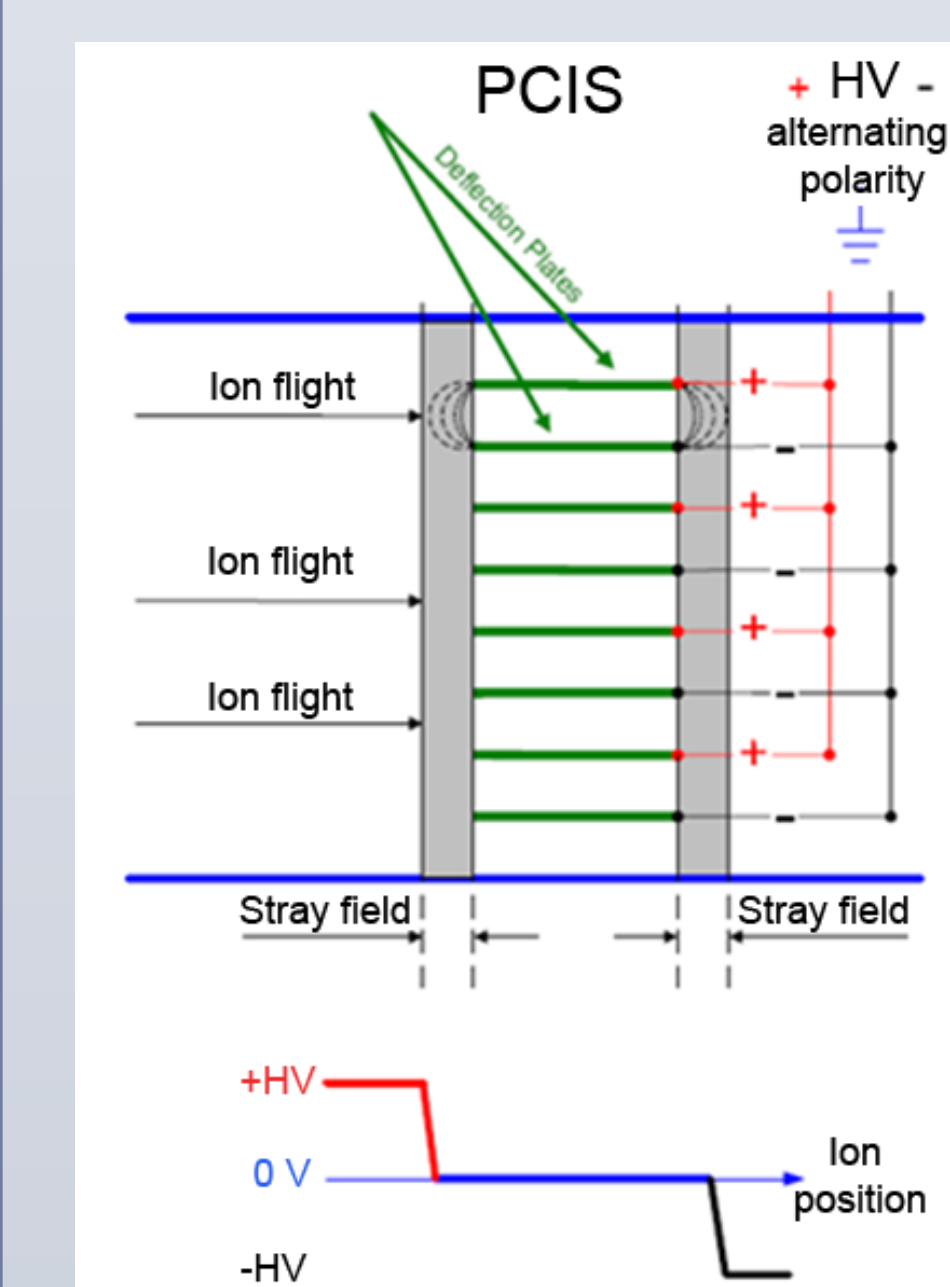
Ions with same  $m/z$  but higher kinetic energy penetrate deeper into reflector electrostatic field, delaying their arrival time at the reflector detector, which results in improved resolution.

Focus in time and space about 30% of difference in kinetic energy. Two stages minimize chemical noise.

## Tandem MS

	Post Source Decay (PSD)	In Source Decay (ISD)
Fragmentation location	After source	Inside source
Precursor ion selection	yes	No
Precursor ion mass	<4 KDa	1-100 KDa

### Post Source Decay (PSD)

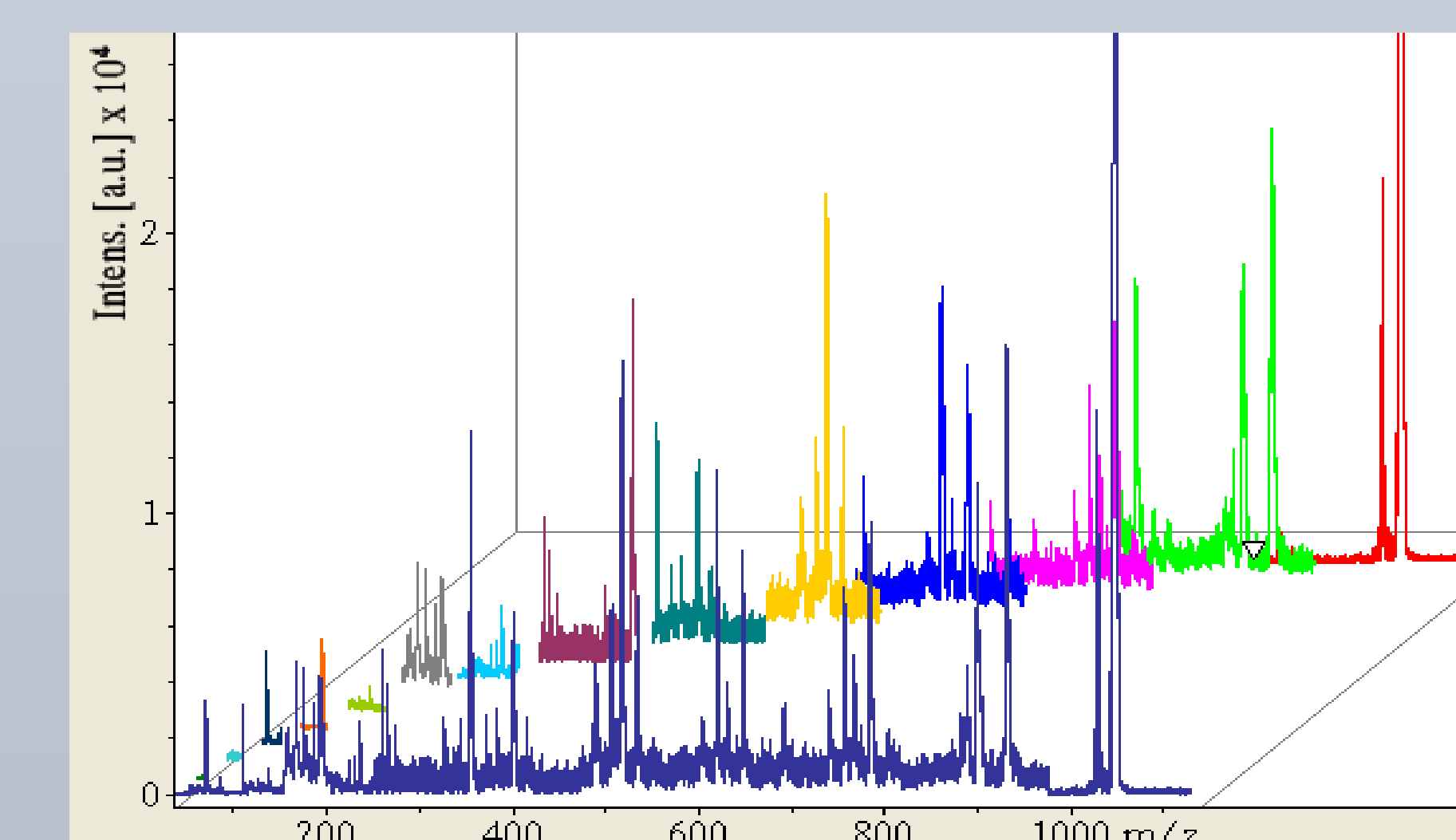


### Precursor Ion Selector (PCIS)

Electrostatic field perpendicular to the ion flight path deflects ions.

5-10 Da window

In **FAST** (Fragmentation Analysis and Structural TOF) method reflector voltage is reduced stepwise to guide fragments to the reflector detector.



Fragments and combined PSD spectra of Angiotensin II peptide