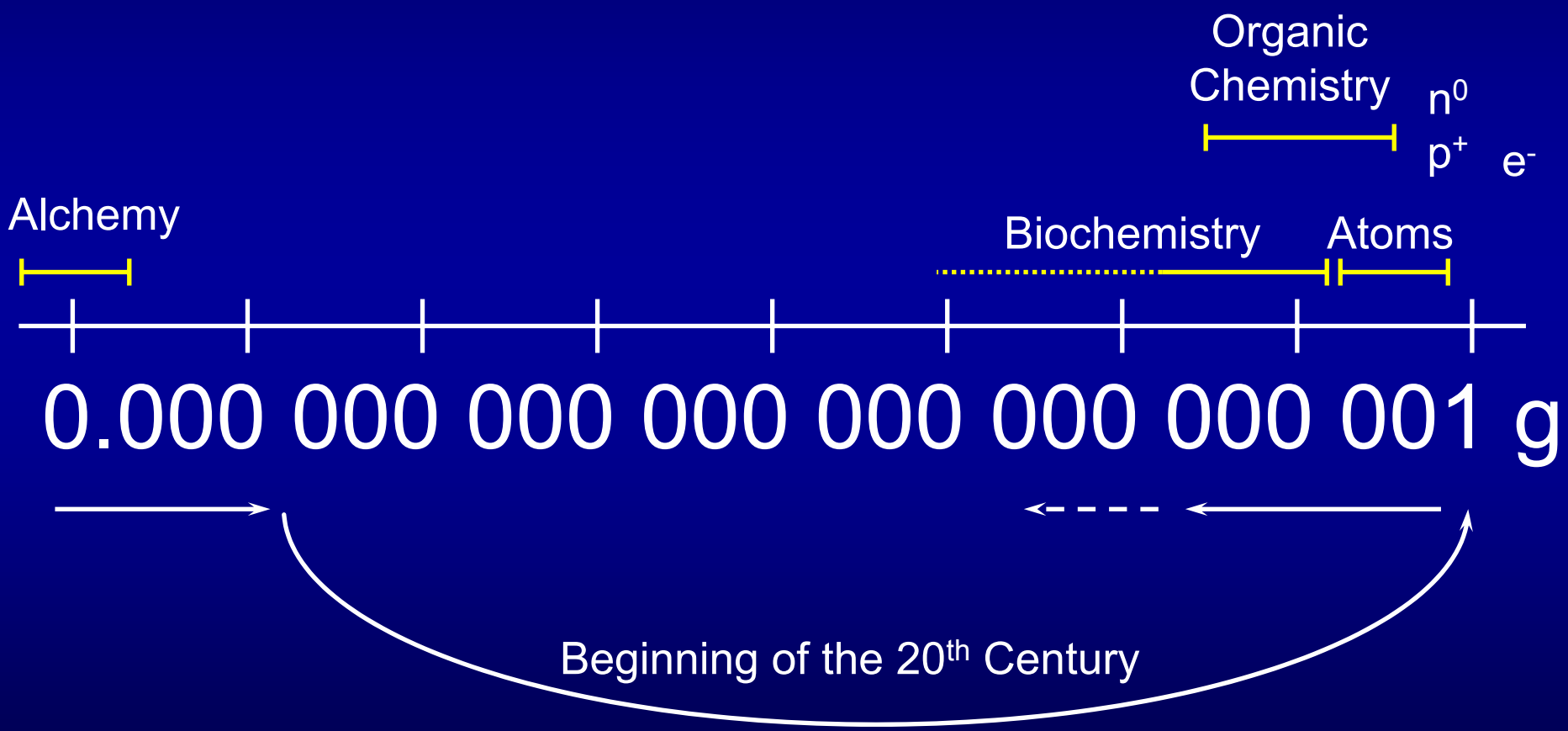
MALDI MS Tutorial

Pierre Chaurand, Lisa Manier

Mass Spectrometry Research Center

Vanderbilt University

Mass Range


Organic
Chemistry
n^0
p^+
e^-
Alchemy
Biochemistry
Atoms
0.000 000 000 000 000 000 000 001 g
Beginning of the 20th Century

Desorption / Ionization : The Probes

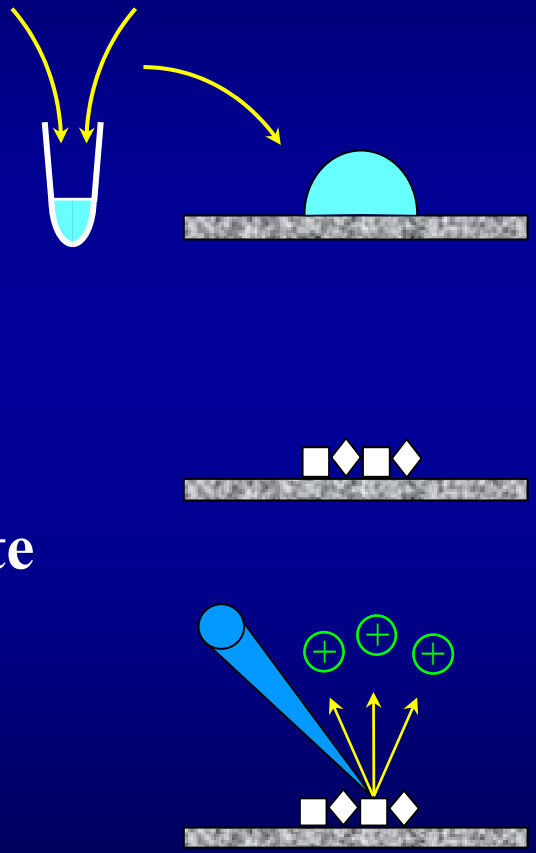
Molecules are brought from a surface into the gas phase (desorbed) and ionized at the same time.

- **Plasma Desorption (PD)**
  ^{252}Cf fission fragments, High energy (MeV) particles or ions
- **Fast Atom Bombardment (FAB)**
  Low energy atoms (keV), (cesium)
- **Secondary Ion MS**
  Low energy ions (keV), (cesium, gallium...)
- **Matrix-assisted Laser Desorption/Ionization (MALDI)**
  UV and IR lasers

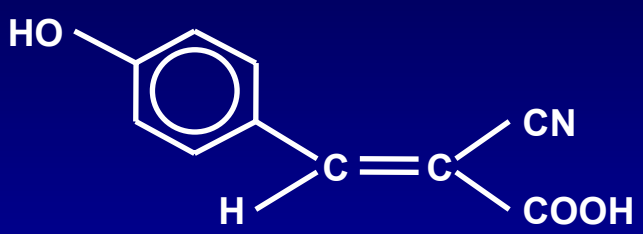
Matrix Assisted Laser Desorption-Ionization or MALDI

- Mixture between a MATRIX and an analyte molecule (5000 for 1)
- Co-crystallization (solid sample)
- Laser pulse ($10^6 W/cm^2$)
- Desorption-Ionization of the analyte molecule (Molecular ion $[M+H]^+$)

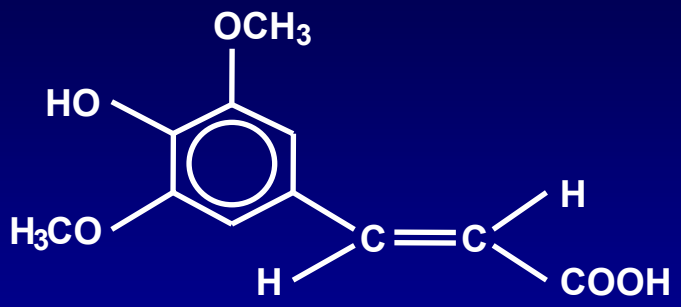
⇨ Analysis of the molecular ions by time-of-flight mass spectrometry



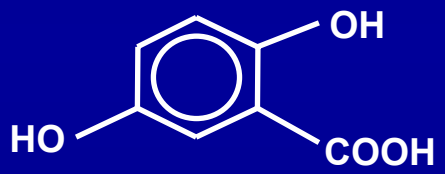
Matrix for UV nm laser light (337 & 355 nm)



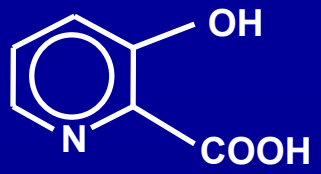
Alpha Cyano-4-hydroxy-cinnamic ac. (αCHCA)



Sinapinic ac. (SA)



2,5-Dihydroxy- benzoic ac. (DHBA)



3-Hydroxy-picolinic (3HPA)

- Absorb at wave length used
- Low vapor pressure (< 10^{-7} Torr)
- Solvent compatible with sample
- Co-crystallize with sample
- Proton donor (M → $[M+H]^+$)

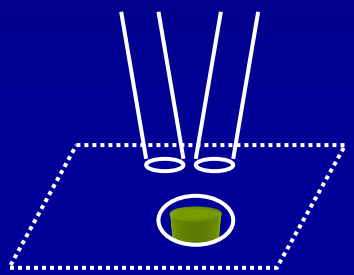
Matrix for UV laser light (337 & 355 nm)

Biomolecule	Matrix	Polarity
Peptides	αCHCA	⊕
Proteins	SA- αCHCA	⊕
Polysaccharides	DHBA	⊕
Nucleic ac.	3HPA	⊖/⊕

SA: 10-20 mg/ml, in 50/50/0.1 - Acetonitrile/H2O/TFA
αCHCA: 10 mg/ml, in 50/50/0.1 - Acetonitrile/H2O/TFA
DHBA: 10 mg/ml, in: 100/0.1 - H2O/TFA
50/50/0.1 - EtOH/H2O/TFA

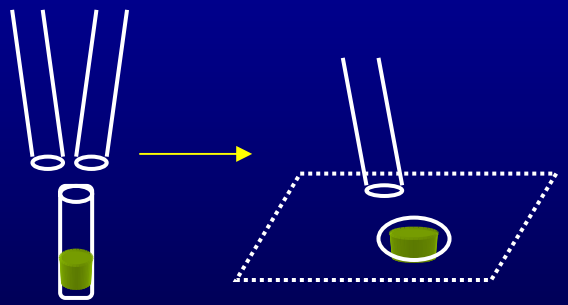
Loading Methods

Droplet method:



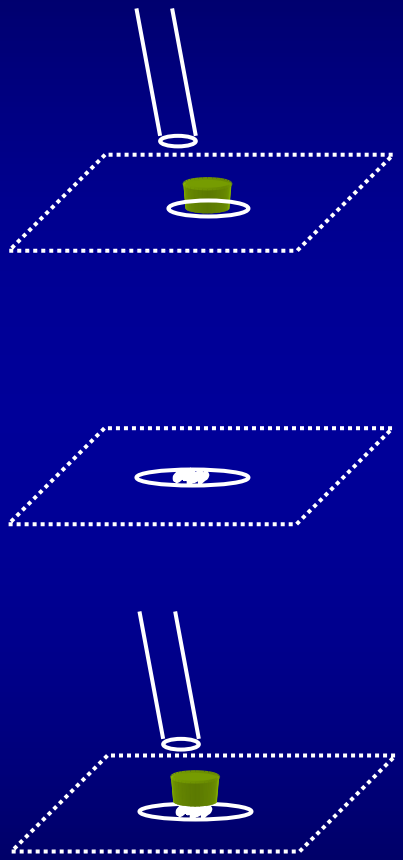
1. Pipette 0.3-0.5 µl of sample directly onto the sample plate.
2. Immediately pipette 0.3-0.5 µl of matrix on top of sample drop.
3. Dry in the desiccator under vacuum.

Mixing method:



1. Mix 0.3-0.5 µl sample and 0.3-0.5 µl matrix solution in a small Eppendorf tube.
2. Pipette ~1 µl of mixture onto the sample plate.
3. Dry in desiccator under vacuum.

Thin Layer Method



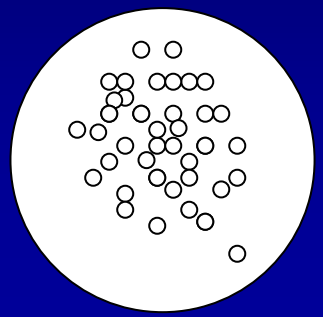
1. Make up a solution of 99% acetone/ 1% 0.1% TFA with 20 mg/ml CHCA or 100% MeOH with 20 mg/ml THAP.
2. Pipette 0.5-1 μl of matrix solution onto sample plate and allow it to spread and dry at room temperature and pressure.
3. For acidic sample solutions (<pH2), add the sample directly on top of dried matrix. For other samples, place 0.5 μl of TFA (0.5-10%) on top of dried matrix and then pipette the sample solution on top of the TFA drop.
4. Dry at room temperature and pressure.

Ref. O. Vorm, et al., *Anal. Chem*. 1994, *66*, 3281.

Matrix Crystallization After Drying

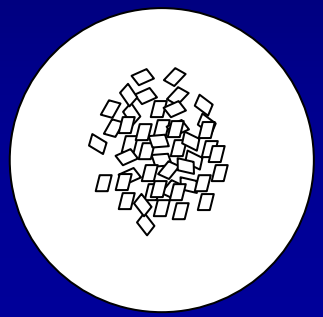
αCHCA

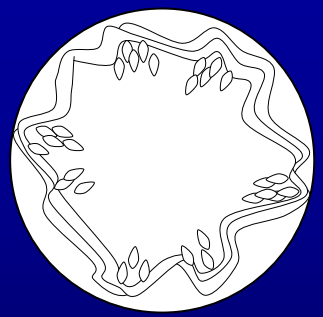
rounded



Sinapinic Acid

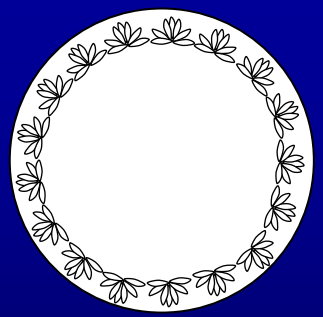
rhomboid





DHBA, air dried

irregular crystals



3-HPA

fan-like around edge of sample well

Buffers

Suitable for Direct Analysis by MALDI MS	Unsuitable for Direct Analysis by MALDI MS
Ammonium bicarbonate	Phosphate buffers
Ammonium acetate	Sulfate buffers
Bis-Tris	Tris (> 100 mM)
Tris (≤ 100 mM)	Hepes (>100 mM)
Hepes (< 100 mM)	

Sample Clean up

For samples containing: Phosphate or sulfate buffers, Salts, Detergents

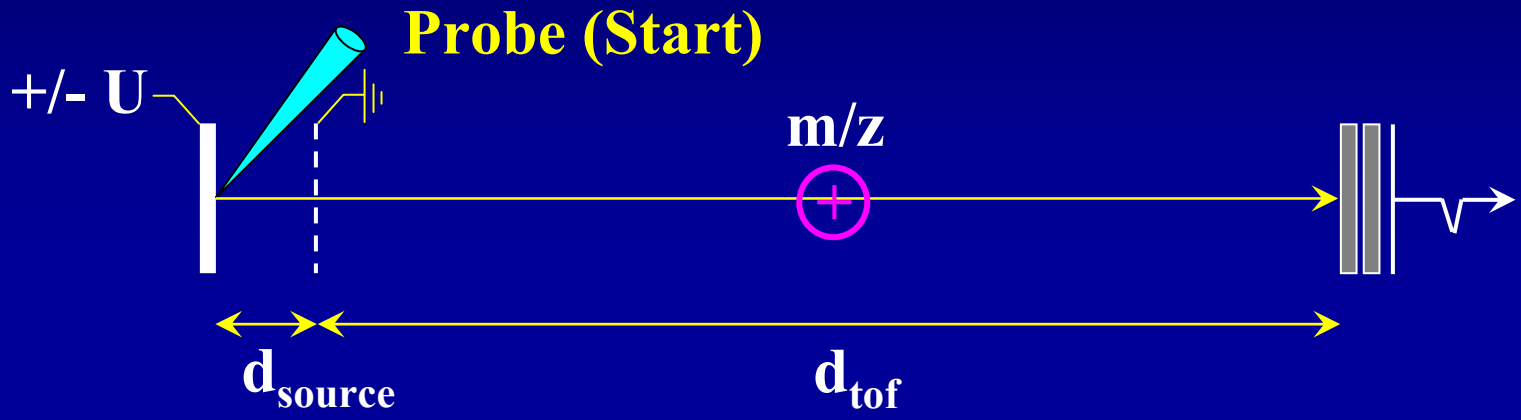
Washing:

1. Use either 0.1% TFA for buffer and salt removal or 5% isopropanol for removal of nonionic detergents.
2. Pipette 1-5 µl of cleaning solvent onto the dried sample and matrix spot. Wait 5-10 seconds and remove the liquid either by pipette or blowing off with a stream of clean air.
3. Repeat step 2 two to five times.
4. Dry before MALDI analysis.

Cation Exchange: Used for desalting

1. Place ~0.1 mg of cation exchange beads (200 mesh) on a piece of parafilm.
2. Add 5 to 10 µl of sample to the beads.
3. Mix by pipetting up and down about 20 times.
4. Allow the beads to settle for 30 seconds.
5. Remove the supernatant with a clean pipet tip and spot onto the MALDI plate.

Time-of-Flight Mass Spectrometry



$$t_{total} = t_{source} + t_{tof}$$

$$\vec{F} = m\,\vec{a} = q\vec{E}$$

Time-of-flight in Source (constant field acceleration)

Force and acceleration: $a = Eq/m$

(a, acceleration; E, electric field in source; q, charge; m, mass)

Velocity and time: $a = dV/dt$

(V, velocity; t, time)

$$V = \int Eq/m = V_o + (Eq/m)t$$

$V_o \sim 0$

$$t = \frac{V - V_o}{E}\ (m/q)$$

Position: $d = \int V\,dt$

(d, position in source)

$$d = d_o + V_o t + \tfrac{1}{2}(Eq/m)t^2$$

$d_o \sim 0$, $V_o \sim 0$

$$t_s = \sqrt{\frac{2d_s\,m}{qE}}$$

Time-of-flight in tube (field-free)

Kinetic energy: $E_{kin} = qU = \tfrac{1}{2}\,mV^2$

(U, accelerating potential)

Drift time : $V = \frac{d_{tof}}{t_{tof}}$ or $t_{tof} = \frac{d_{tof}}{V}$

$$t_{tof} = \frac{d_{tof}}{V} = d_{tof}\sqrt{\frac{m}{2qU}}$$

Total time-of-flight

$$t_{tot} = t_s + t_{tof}$$

$t_s \sim 0$

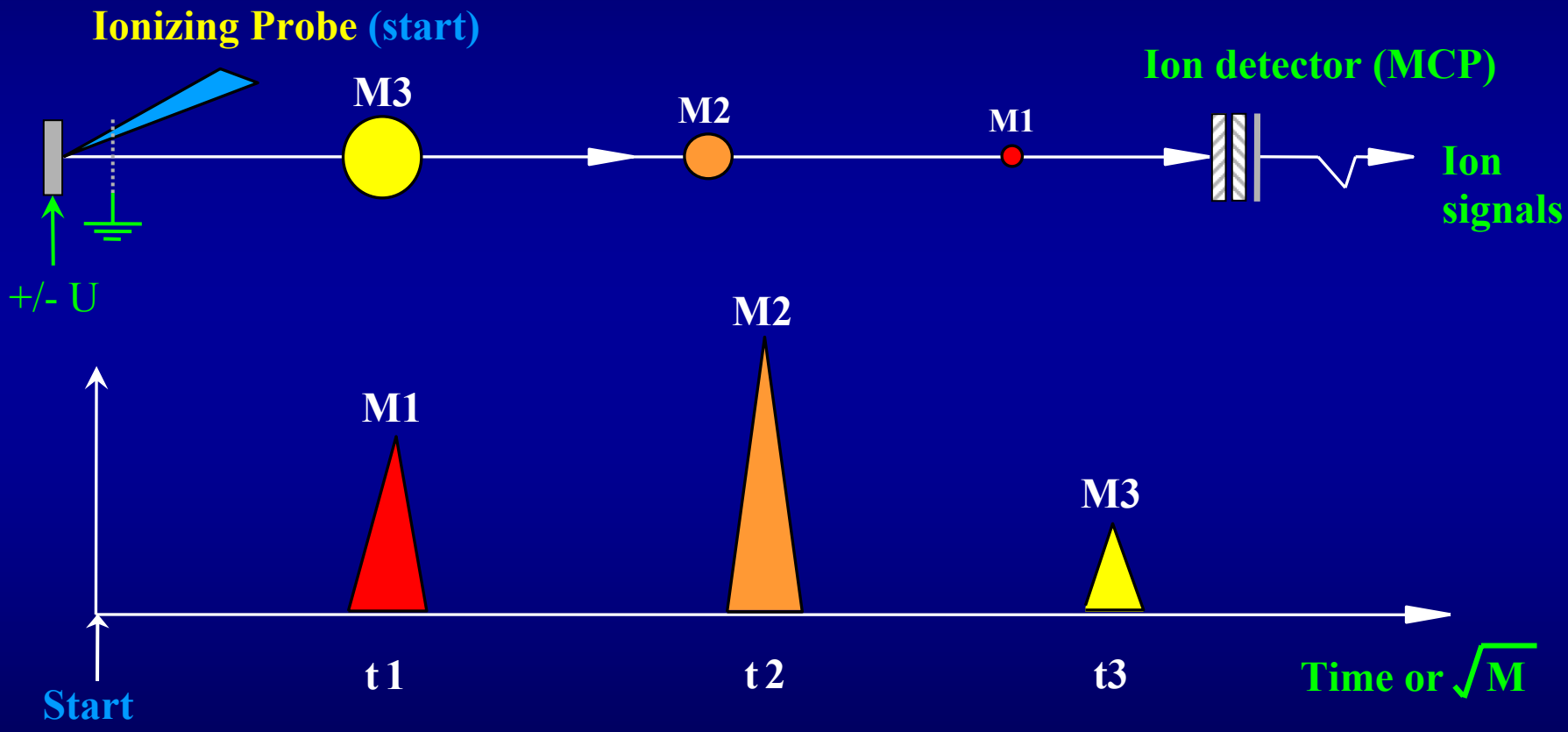
$$t_{tot} = \sqrt{\frac{2d_s\,m}{qE}} + d_{tof}\sqrt{\frac{m}{2qU}}$$

$$t = a\sqrt{m/q} + b$$

where ***a*** and ***b*** are defined by the physical dimensions of the instrument and the operating parameters.

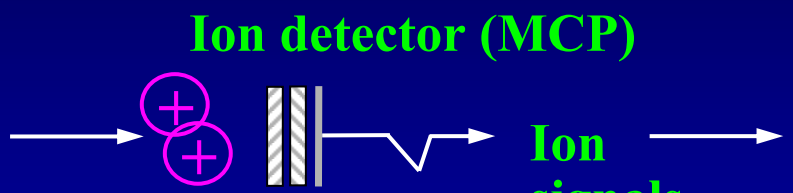
Time-of-Flight Mass Spectrometry (TOF-MS)

Linear TOF :

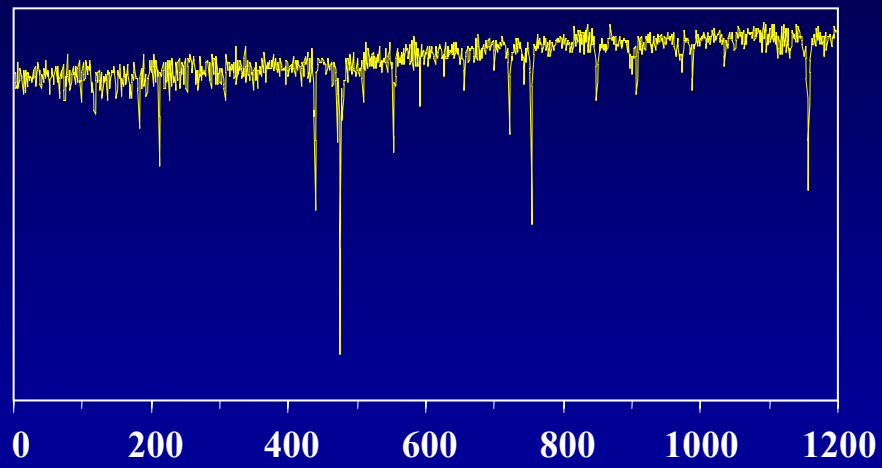


$$t = a\sqrt{M} + b$$

MALDI: Data acquisition

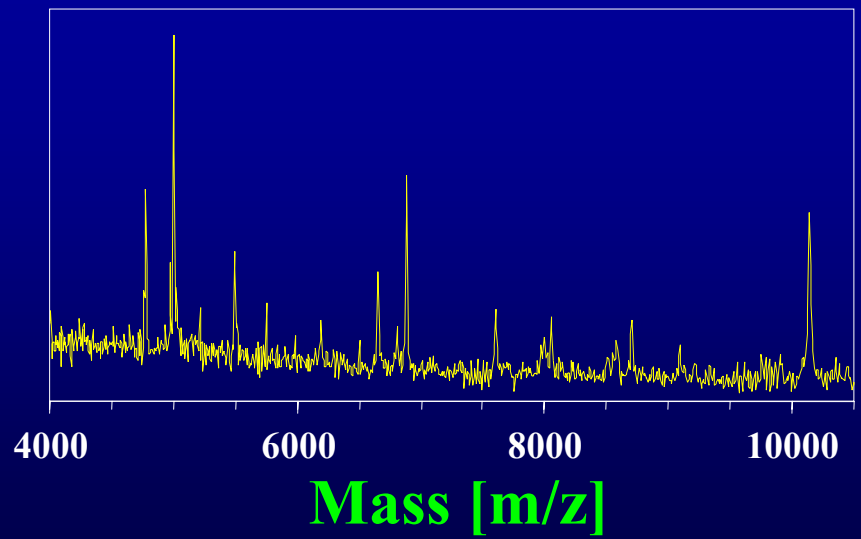


Transient Recorder



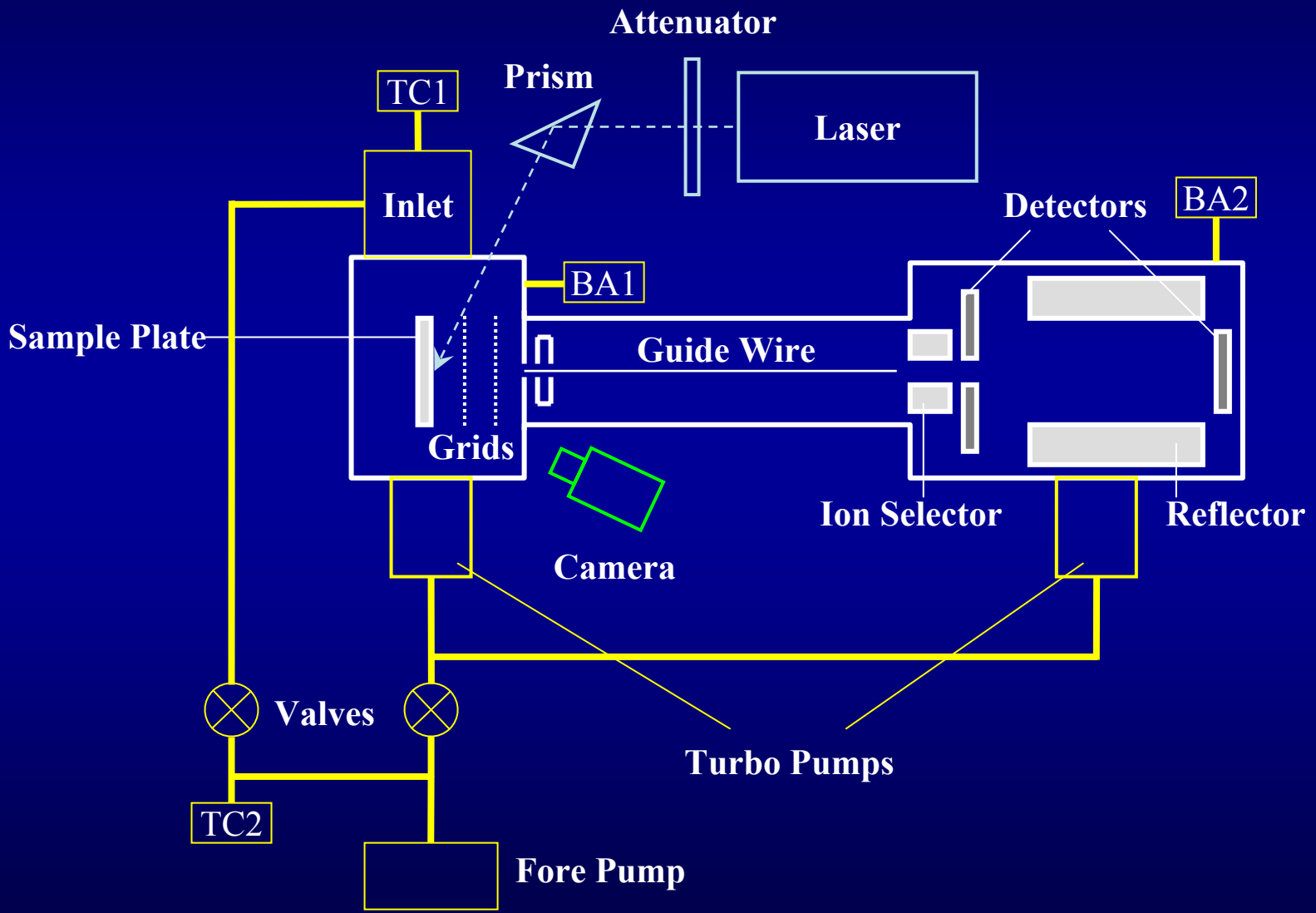
Data transfer to PC,
Calibration

Software



MALDI DE-STR TOF MS from Applied Biosystems

Time-of-Flight Mass Spectrometers, General Organization

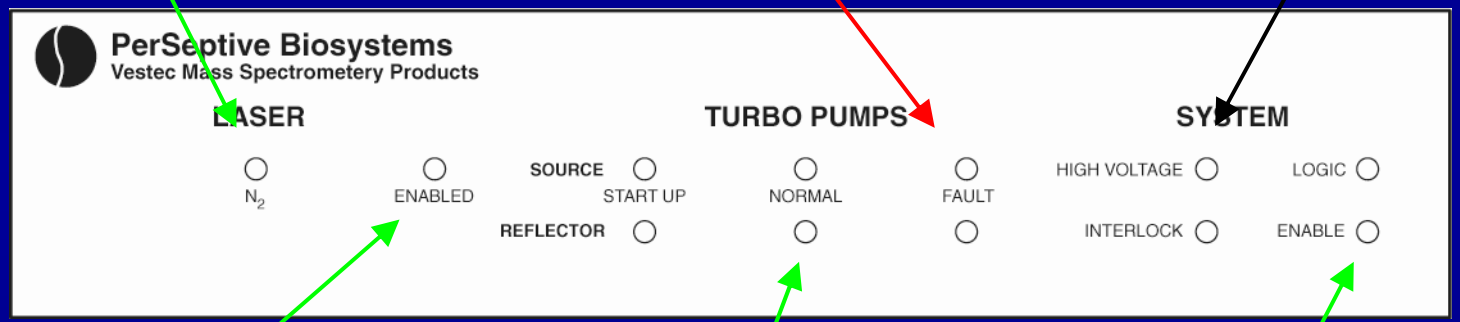


Instrument Panel

Indicates that the laser is powered on

Mayor problem!

High voltage is on

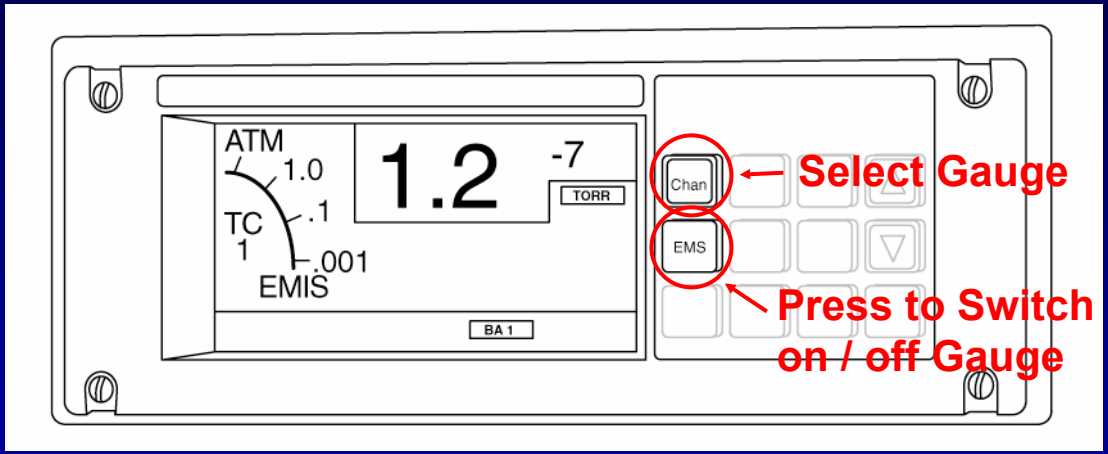


Indicates the laser is firing

Pumps are operating normal

Computer is controlling the instrument (software is ready)

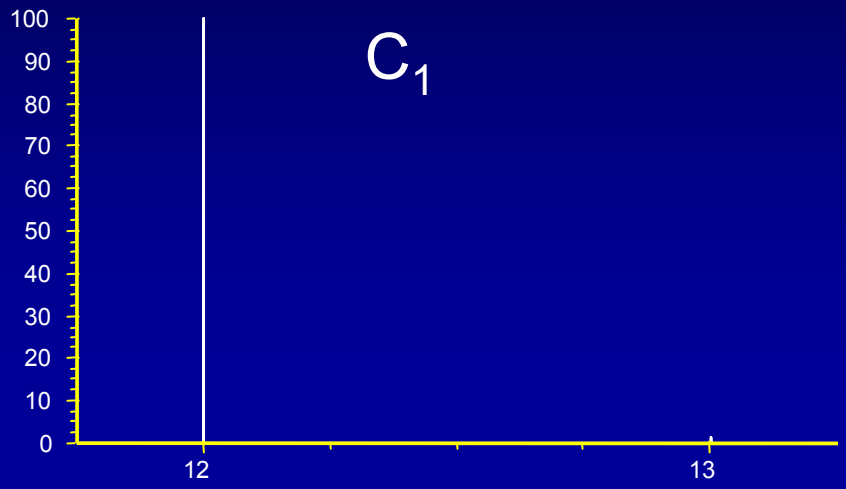
Pressure Gauges

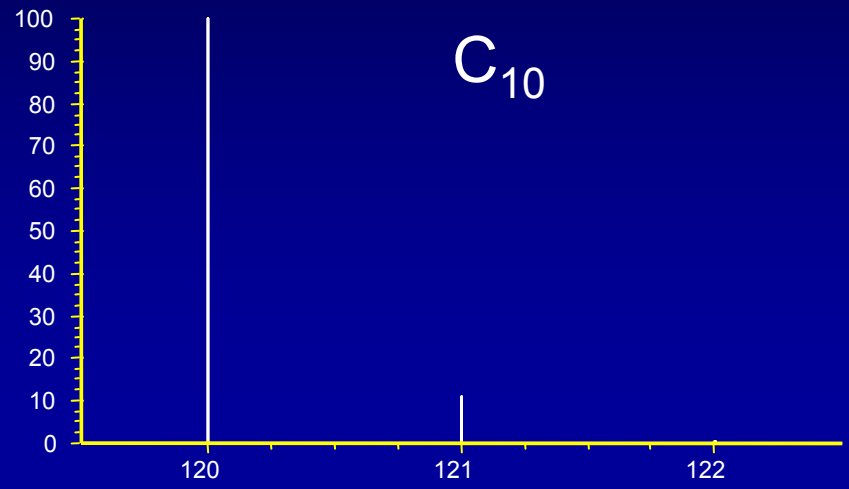


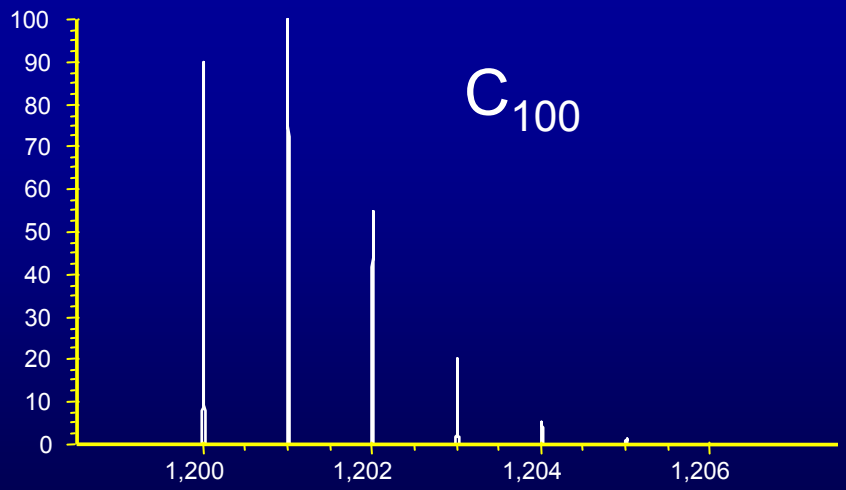
Gauge	Measures	Expected Pressure
BA1	Pressure in main source chamber	Less than 5 x 10 –7
BA2	Pressure in mirror chamber	Less than 5 x10 -8
TC2	Pressure in sample loading chamber	Less than 10 –2 during operation Higher when loading or ejecting sample plate.
TC1,TC3, TC4	Not used	displays E03 (indicates gauge not connected)

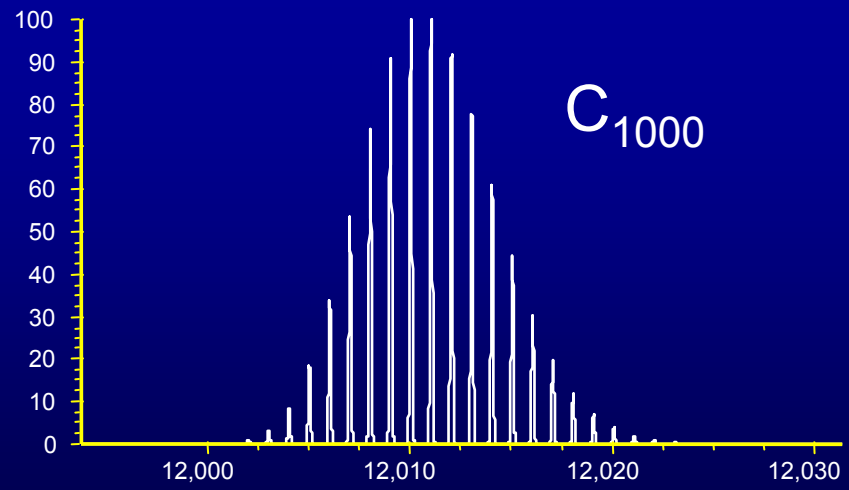
[torr]

Isotopic Distribution







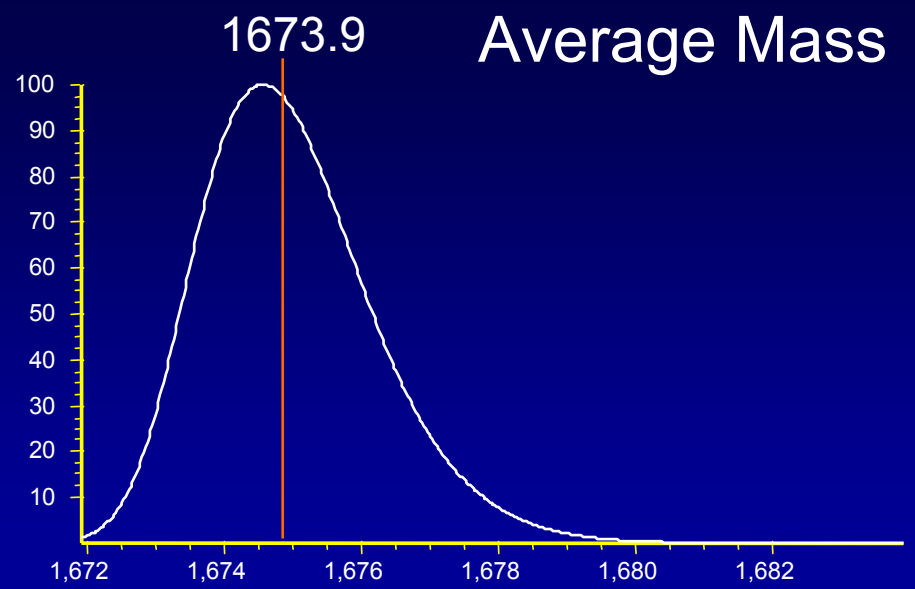


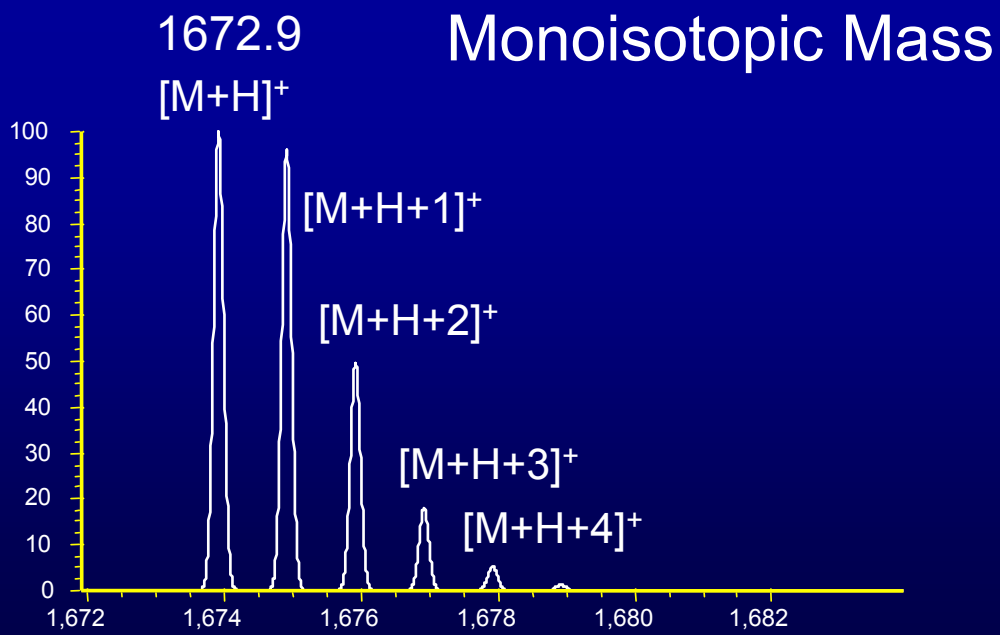
Neurotensin

Res. 1’000

pQLYENKPRRPYIL
MW 1672.9

Res. 10’000





Constant field extraction TOF MS

Single ion counting (PDMS, SIMS, FAB...)

LD, MALDI

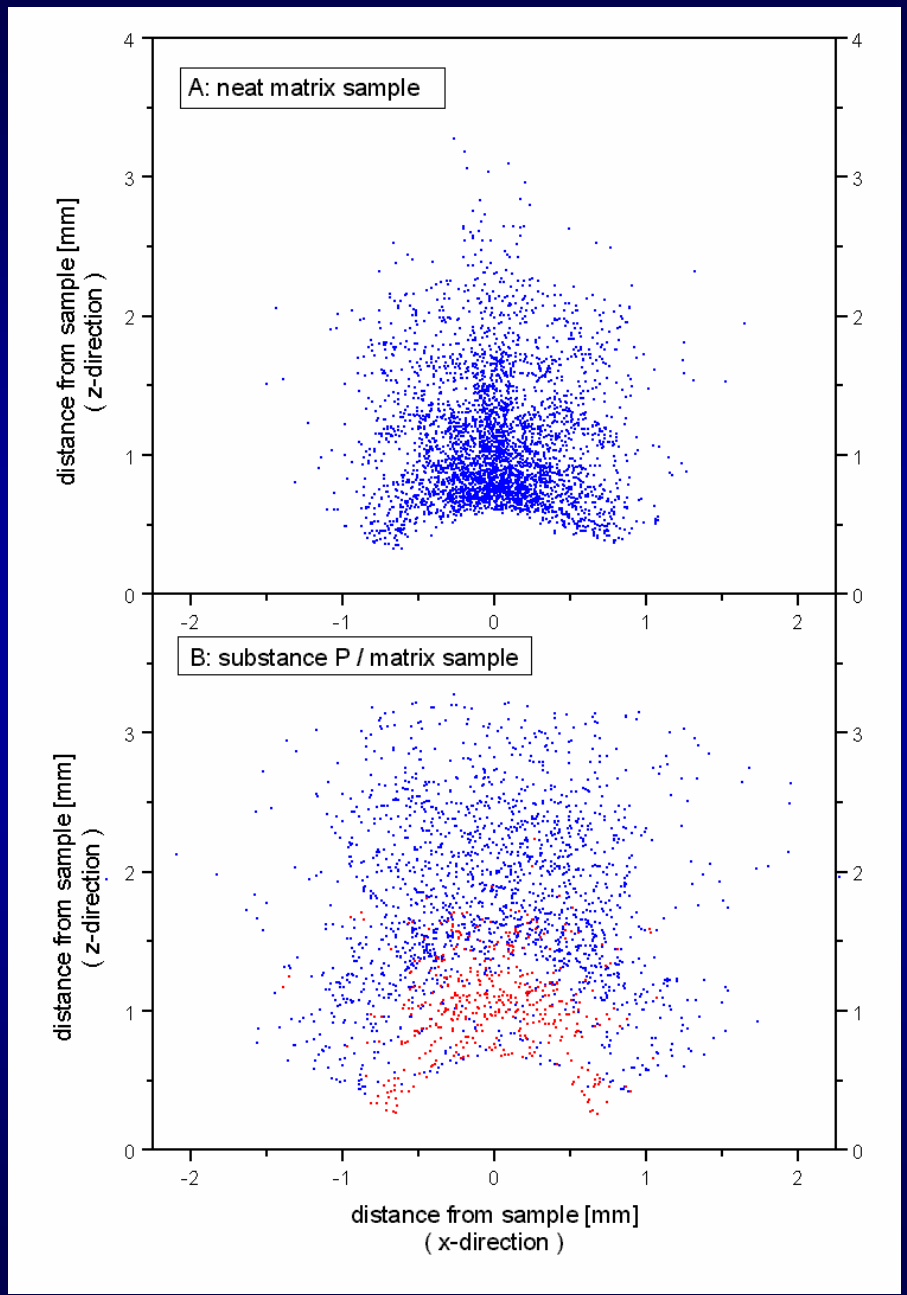
MALDI ion plume

DHB matrix and Substance P $[M+H]^+$ ions densities measured 1 μs after laser impact.

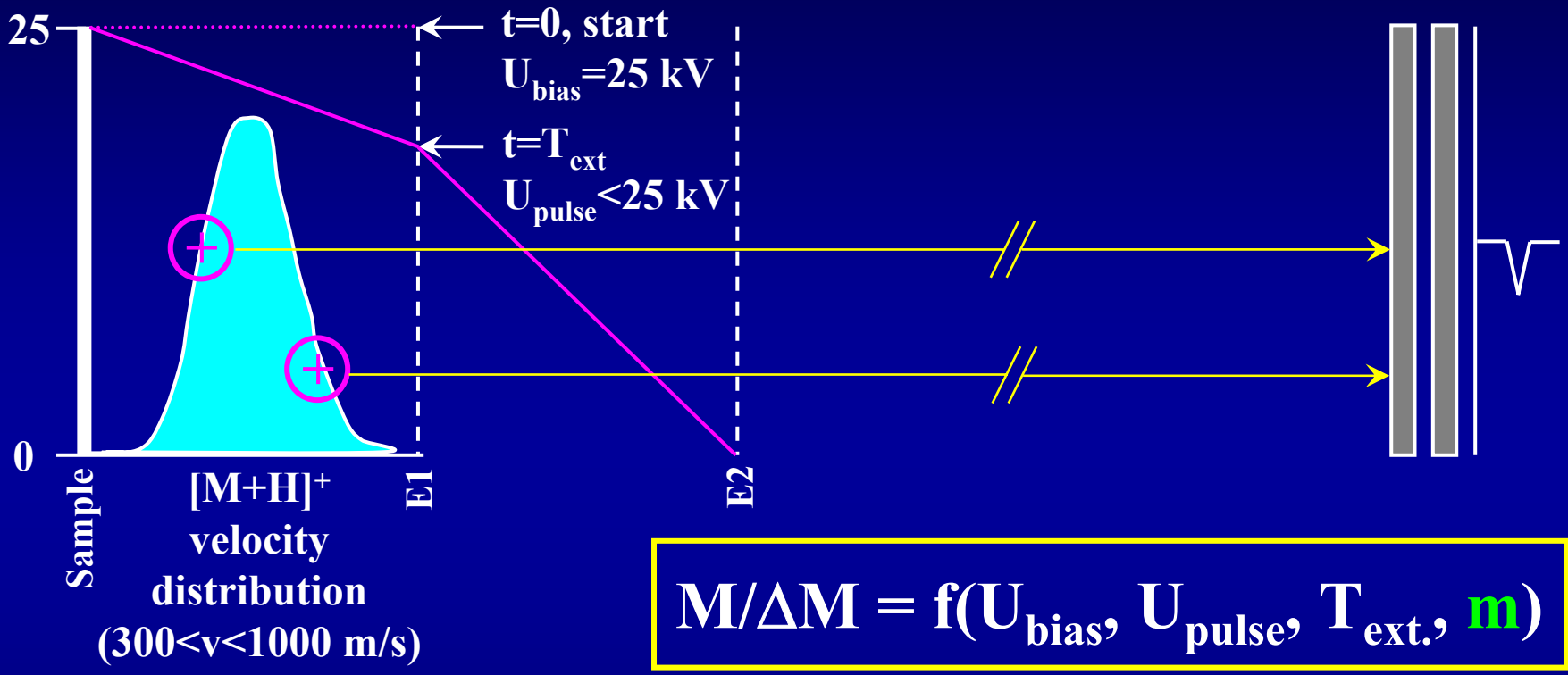
Bleu: DHB
Red: Substance P (MW 1347.7

V. Bokelmann, B. Spengler, R. Kaufmann, Eur. Mass Spectrom. 1, 81-93 (1995)

B. Spengler
J. Mass Spectrom., 32, 1019-1036 (1997)



Delayed Extraction in MALDI TOF MS



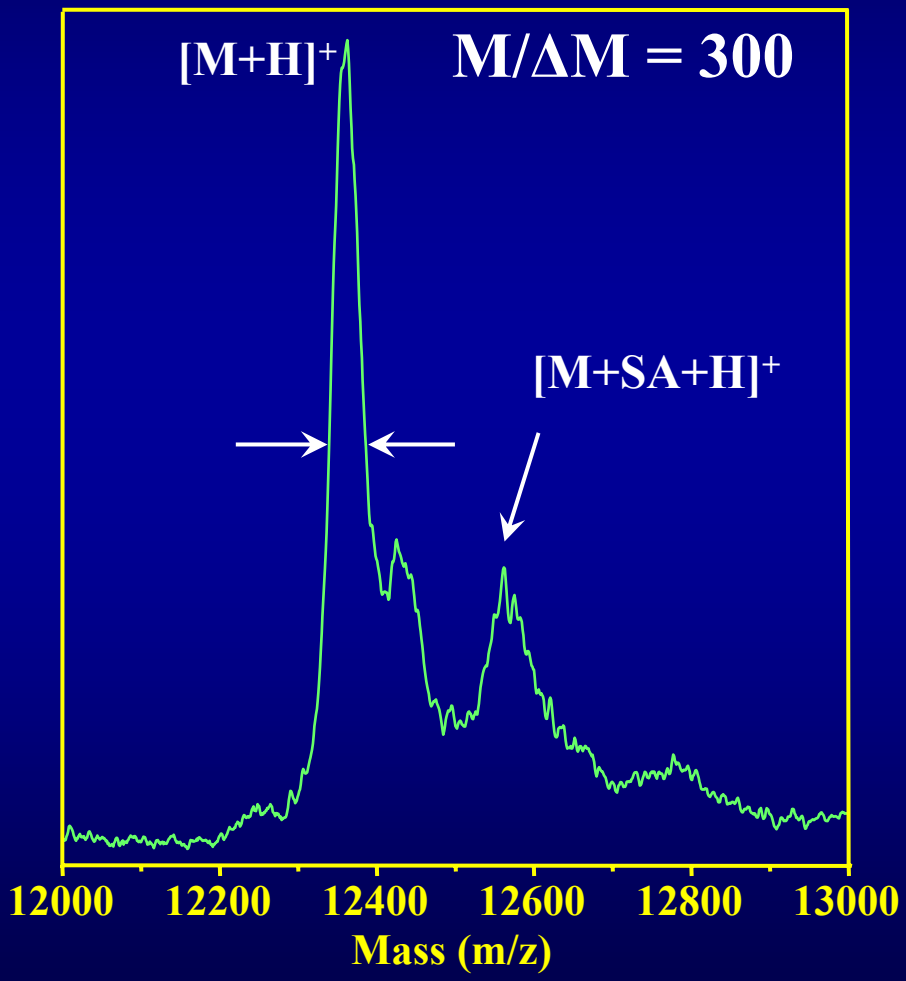
$$M/\Delta M = f(U_{bias}, U_{pulse}, T_{ext.}, m)$$

- Compensation of the initial velocity distribution resulting from the MALDI desorption process to time focus the ions on the detector.

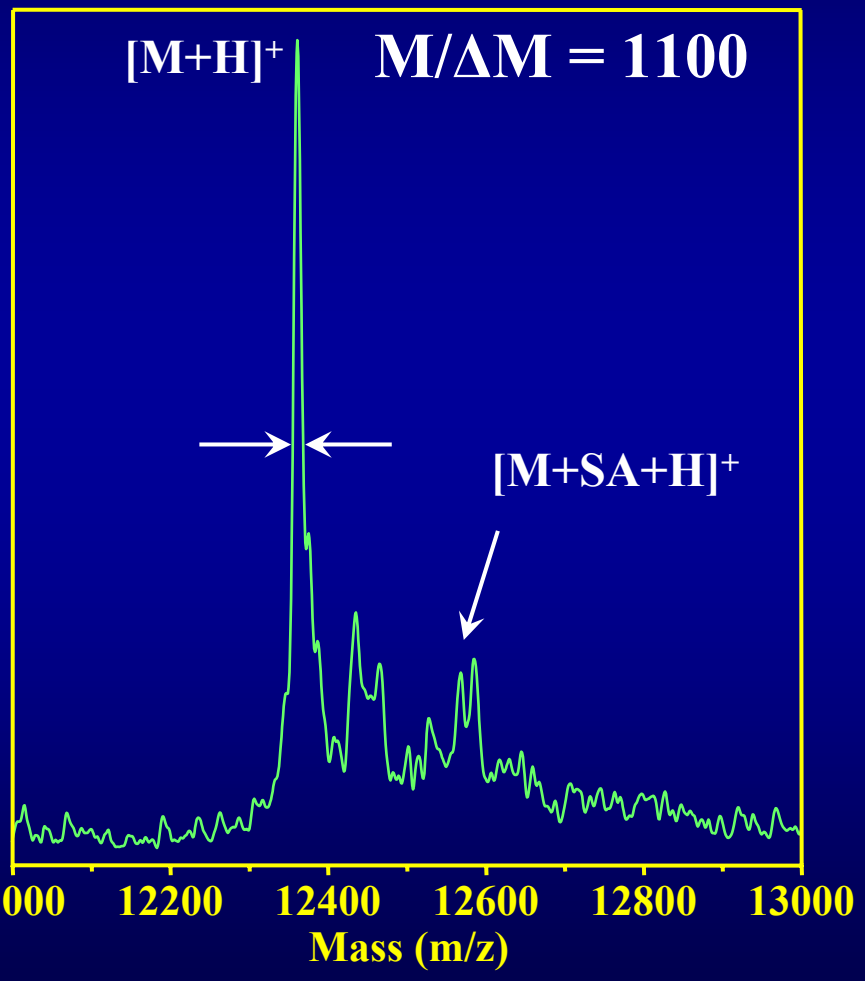
⇒ Increase in resolution

MALDI TOF MS of Cytochrome C, MW 12360.1

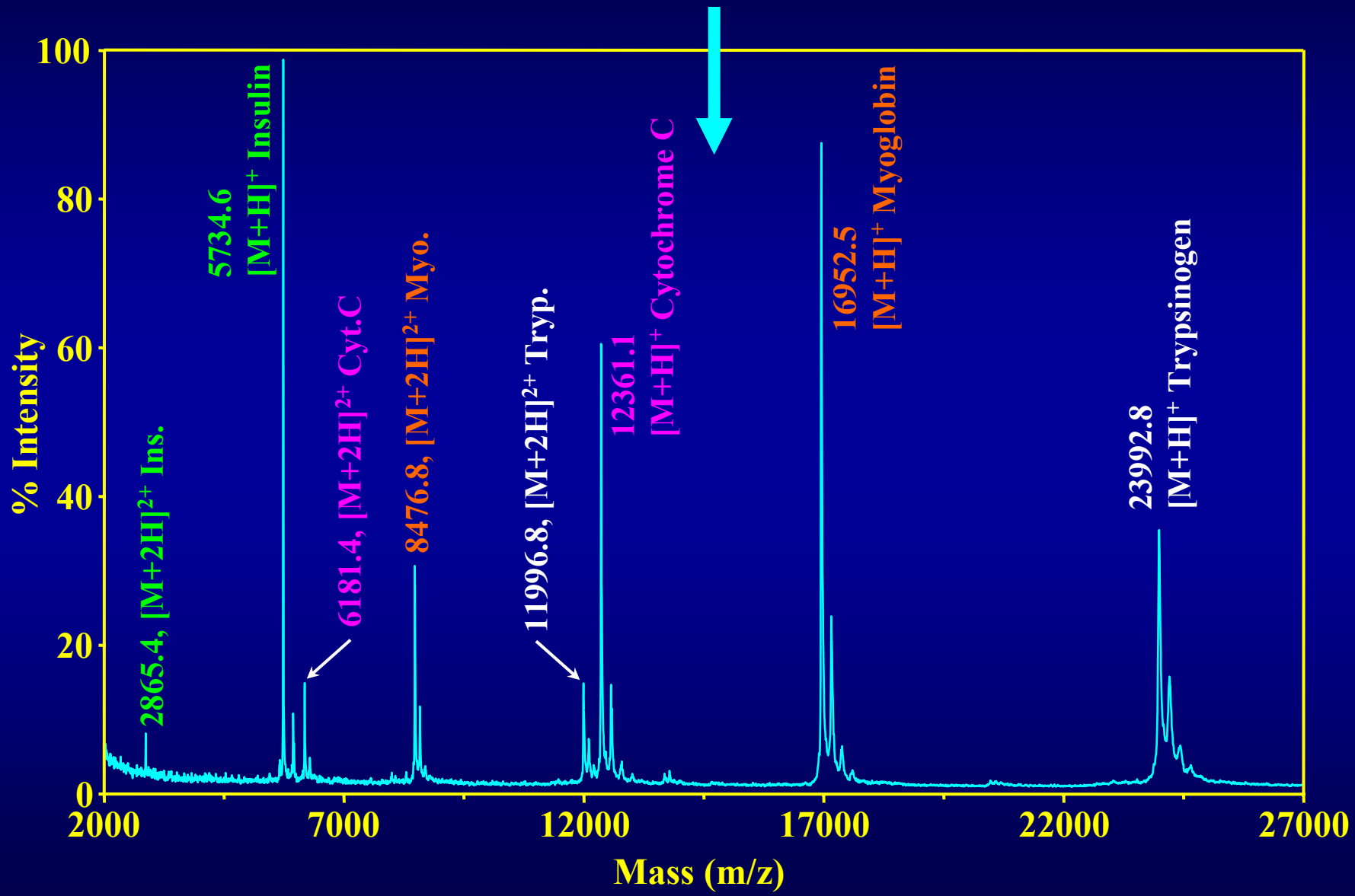
Constant field extraction



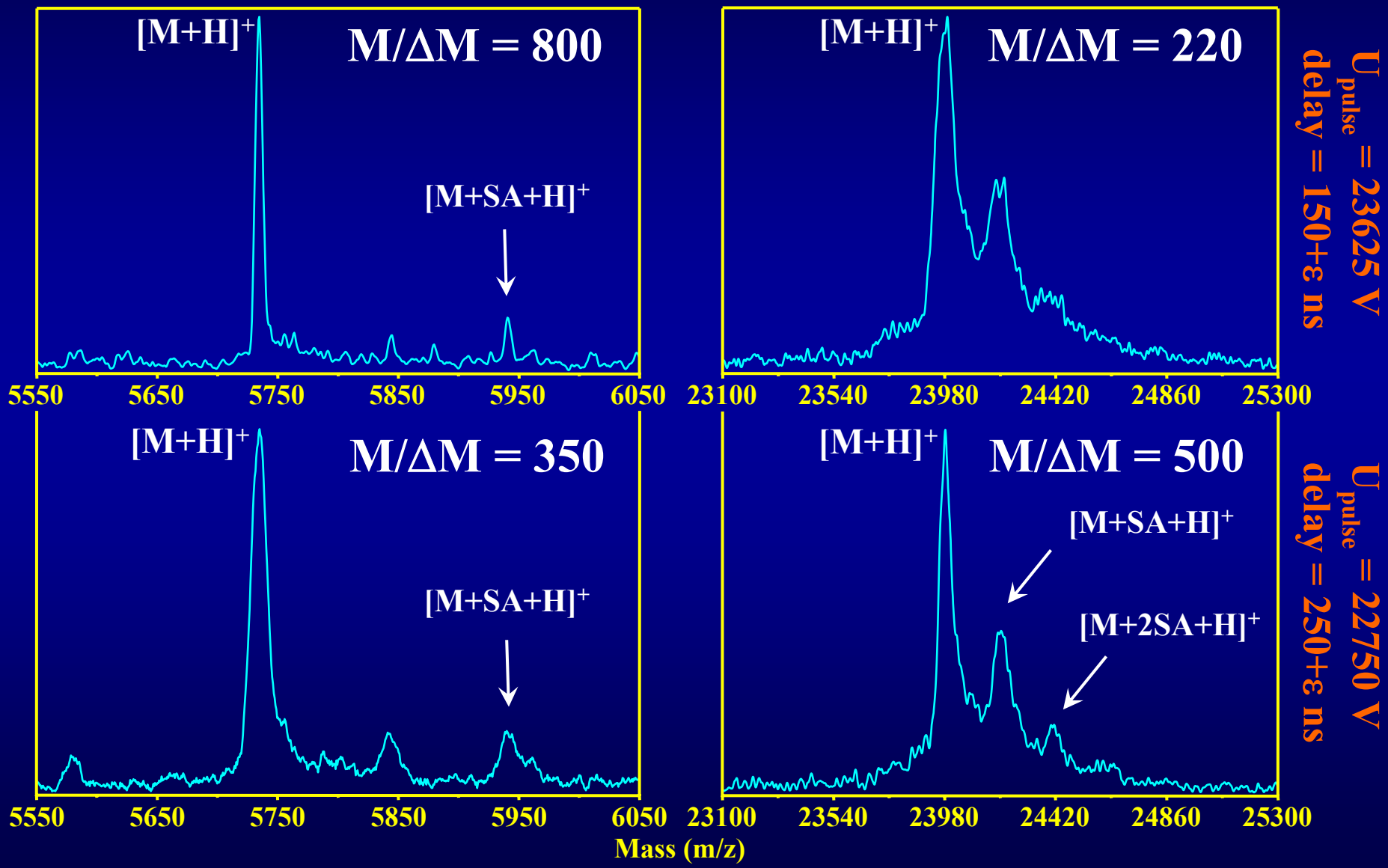
Delayed extraction



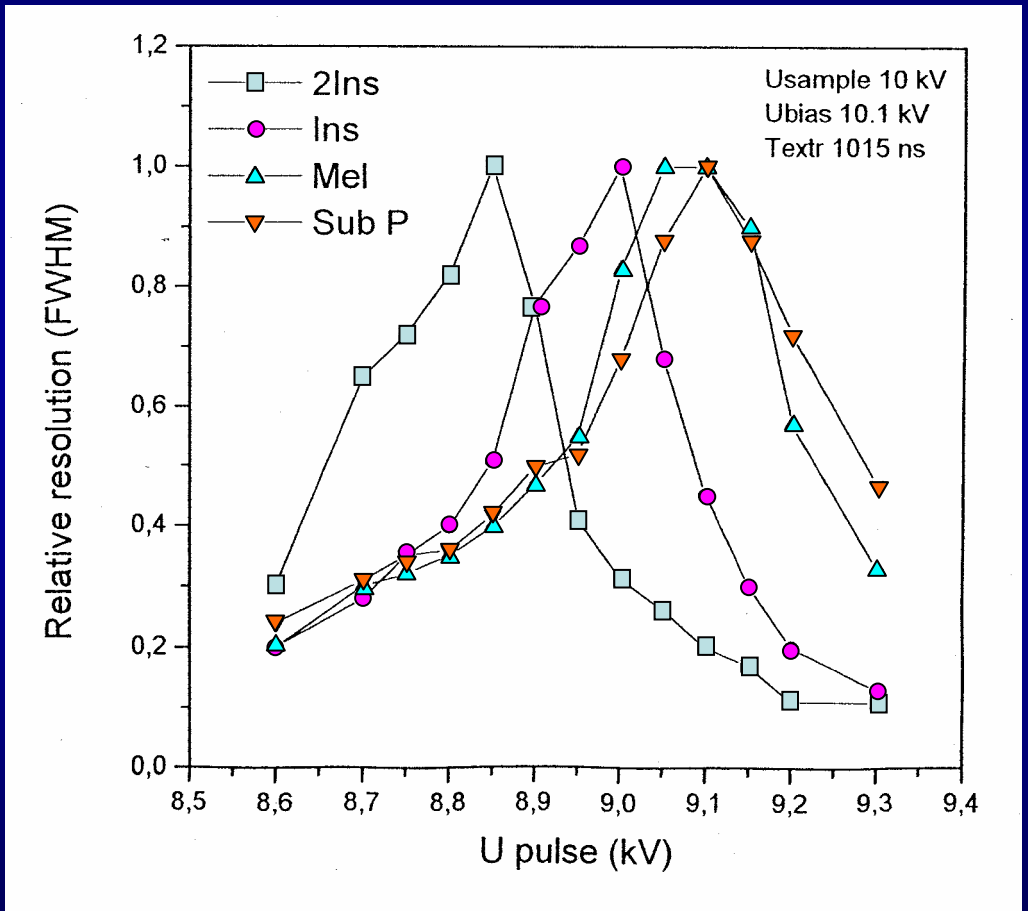
MALDI TOF MS of Protein Mixture



MALDI TOF MS of Protein Mixture, U_{bias} = 25 keV



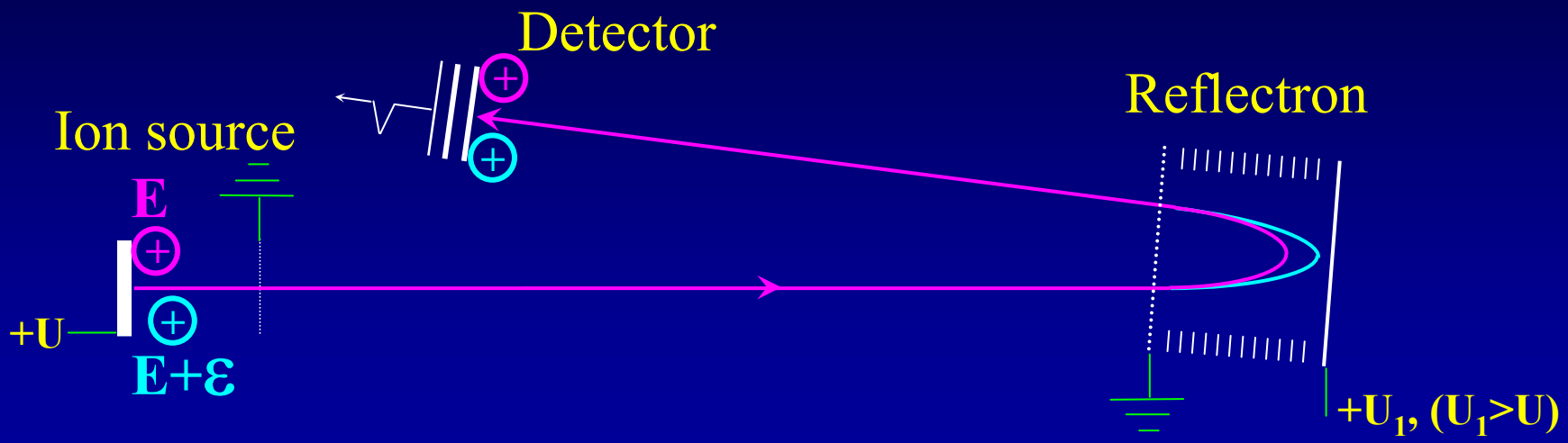
Delayed extraction in linear MALDI TOF MS



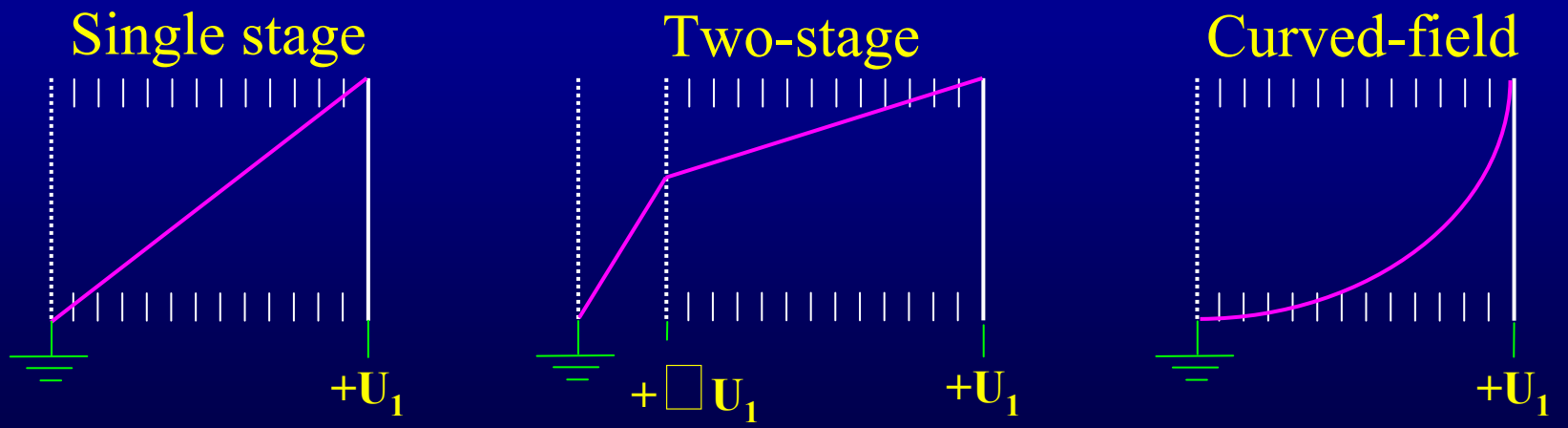
U_{sample} = 10 kV
T_{ext} = 1050 ns

Sub P, MW 1347
Melittin, MW 2848
Insulin, MW 5734
2Ins., MW 11467

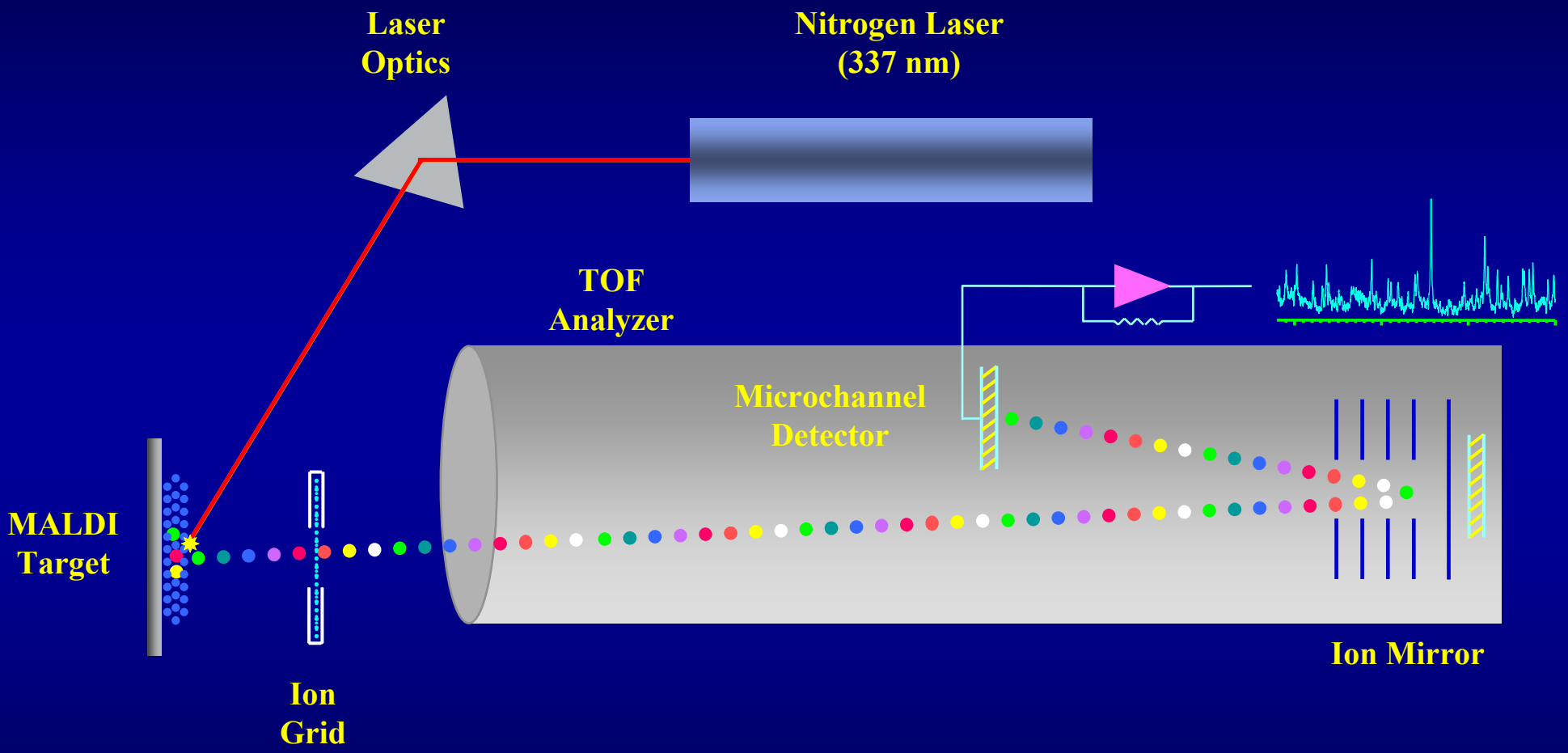
Reflex Time-of-Flight Mass Spectrometry



- **Increase in resolution**
- **Tandem mass spectrometry**

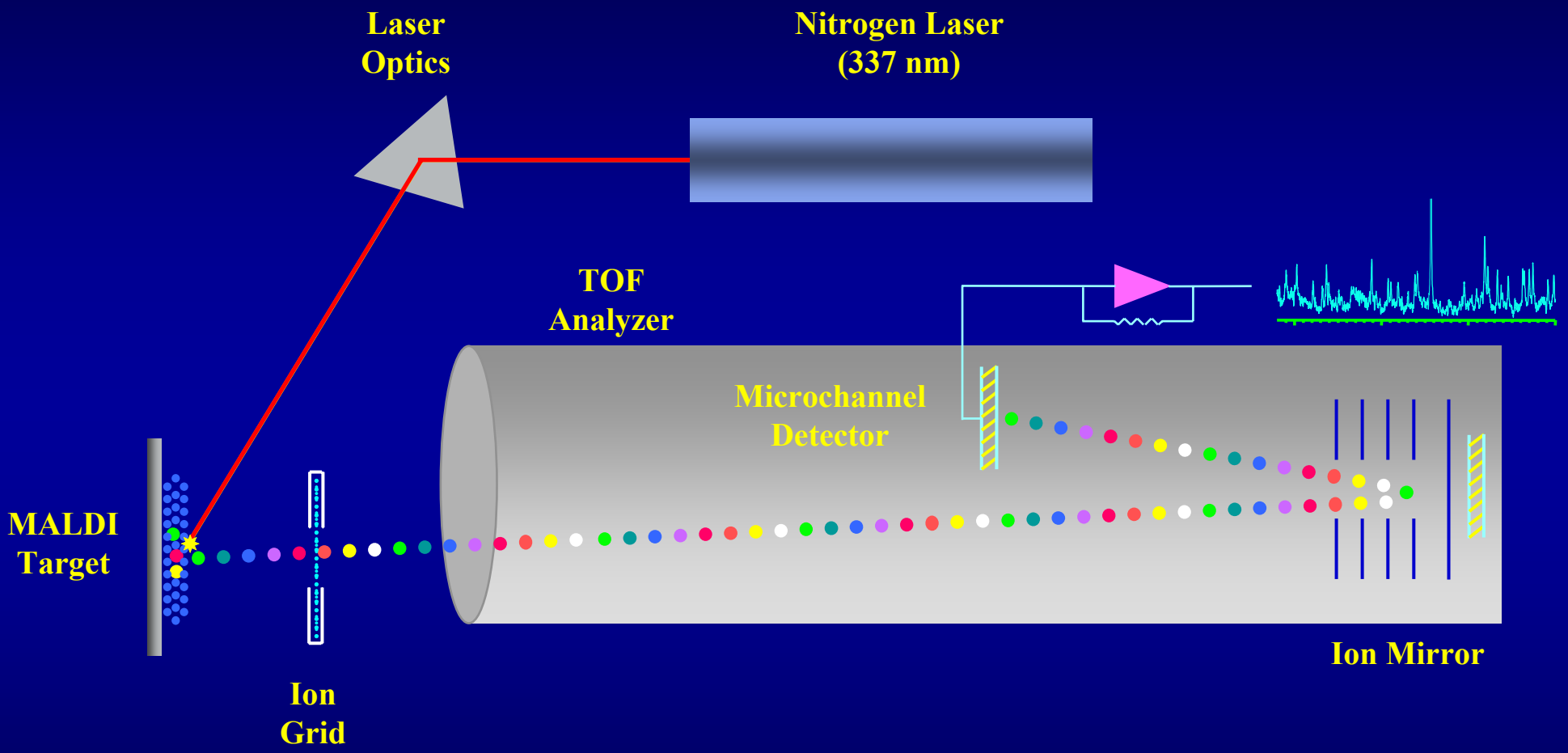


Reflex MALDI TOF Mass Spectrometer



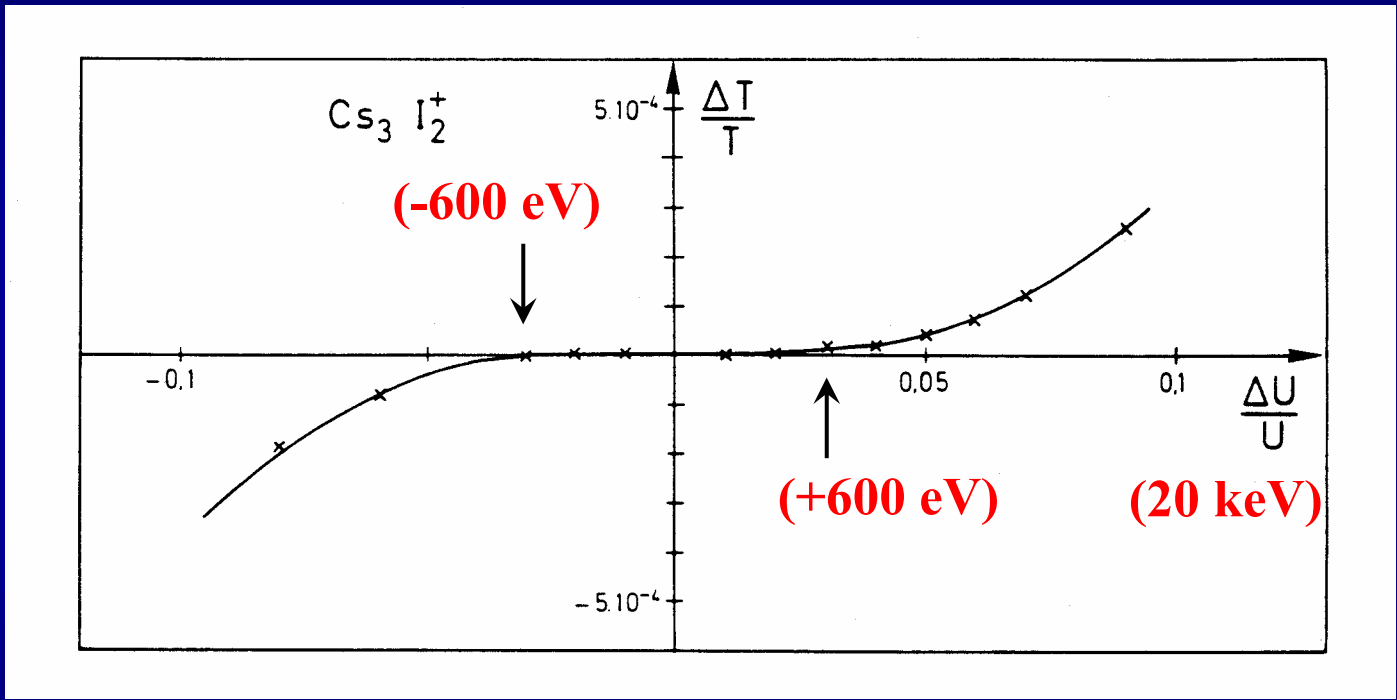
(1)

Reflex MALDI TOF Mass Spectrometer



Time-of-Flight variation f(energy variation)

Two-stage electrostatic reflectron



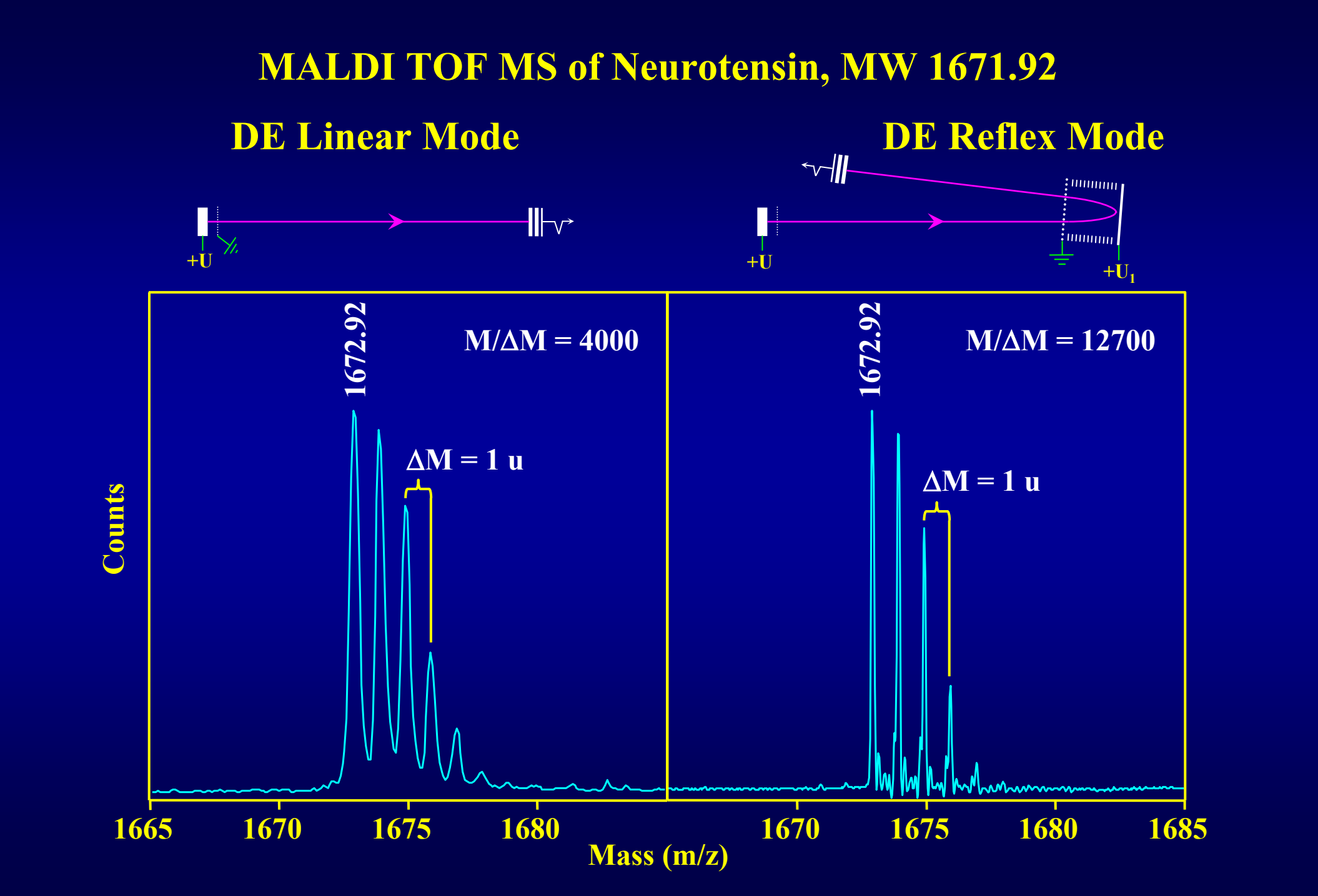
U, Nominal energy

ΔU, Energy variations acquired during the desorption process

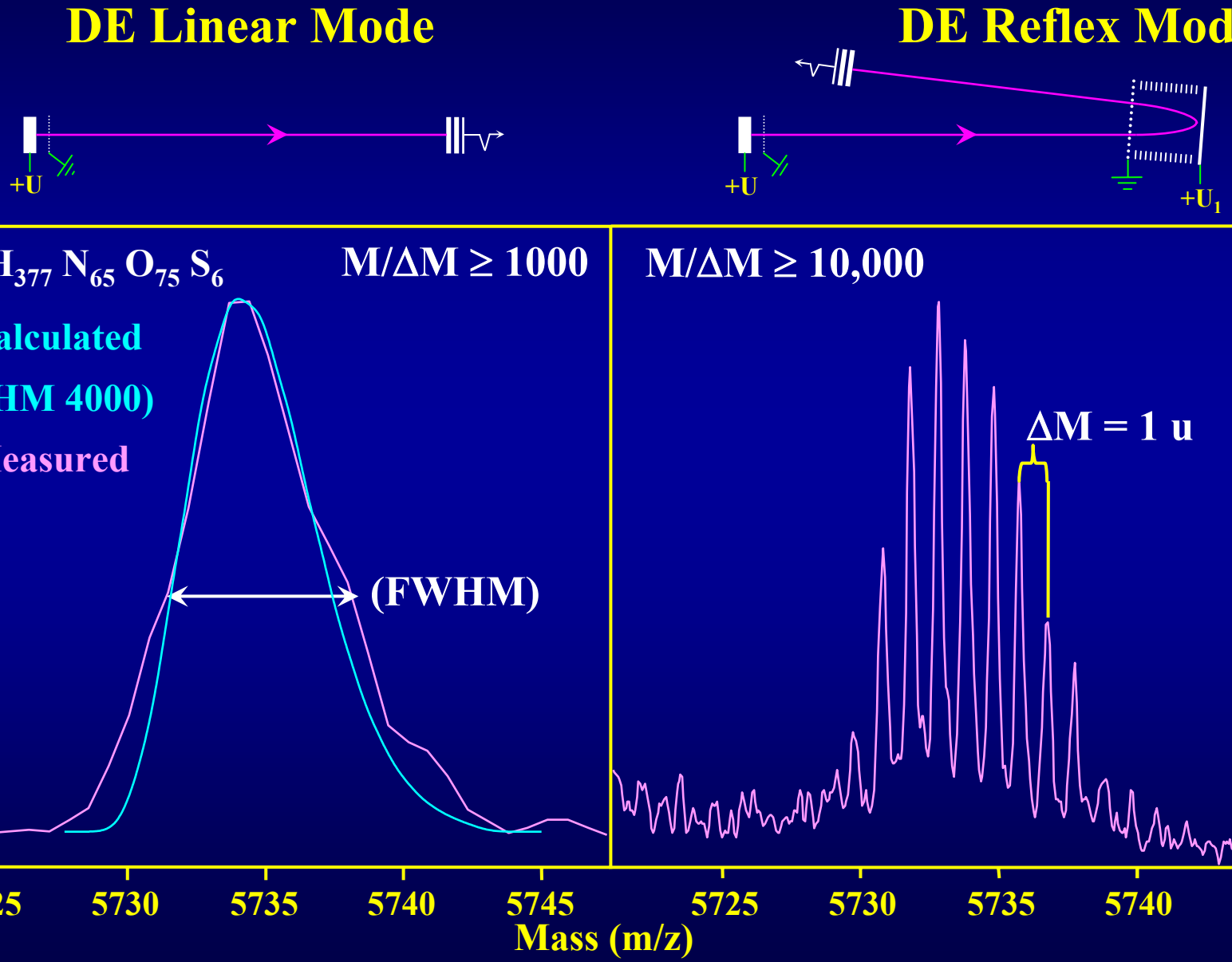
T, Nominal time-of-flight

Δ T, Time-of-flight variations

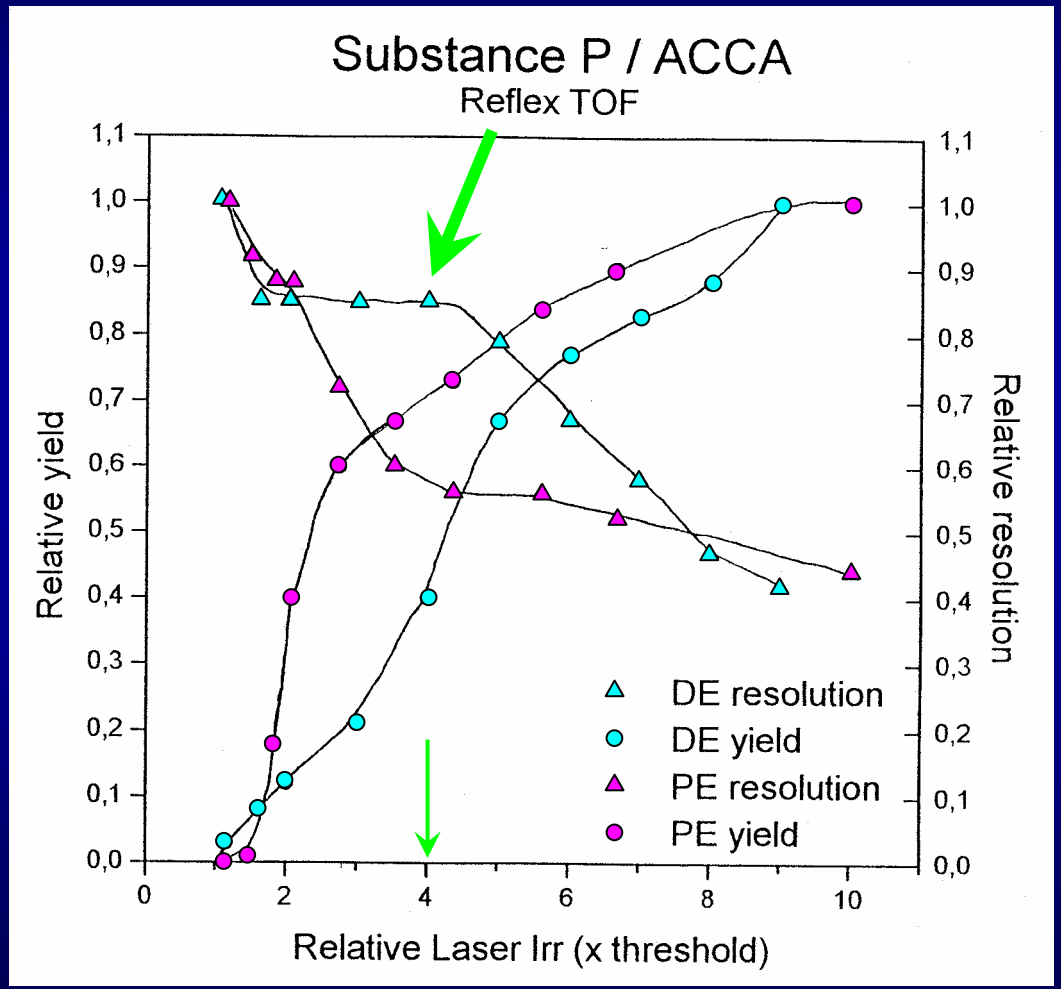
MALDI TOF MS of Neurotensin, MW 1671.92

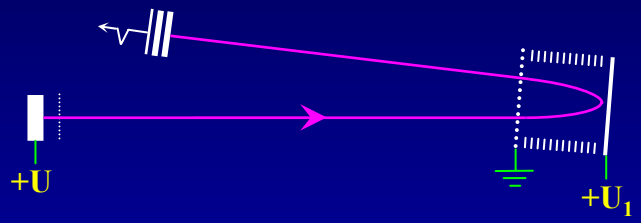


MALDI TOF MS of Bovine Insulin, MW 5733.5



Delayed extraction in reflex MALDI TOF MS

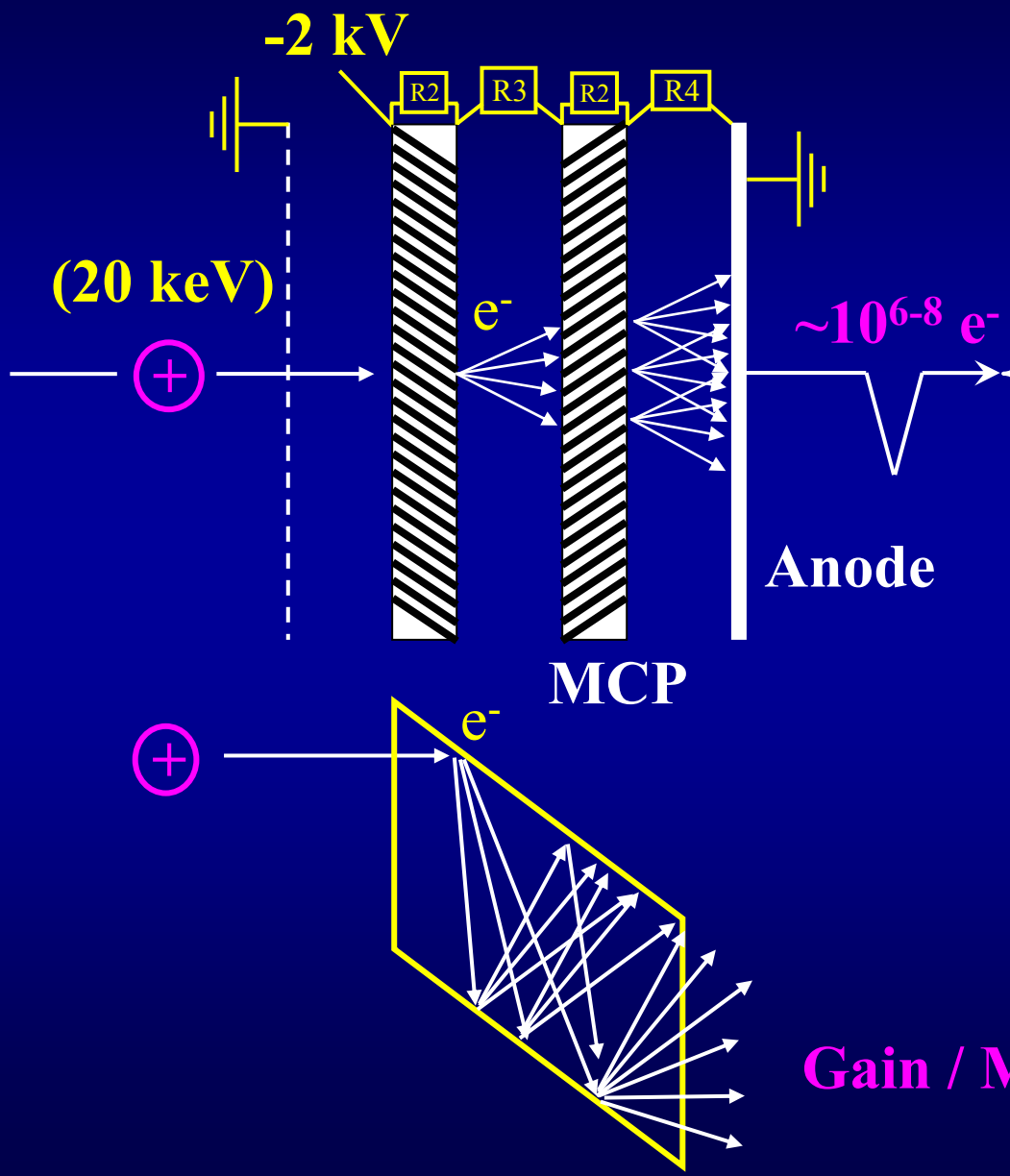




Sub P, MW 1182

Laser irradiance less critical under DE conditions

Ion detection: Microchannel plate detector



Single Ion counting, n < 1

(PDMS, SIMS…)

Time to Digital Converter
+ Histogram PC card

1 < Nb ions < 10

TDC + charge coder,
or Multi Anode Detector

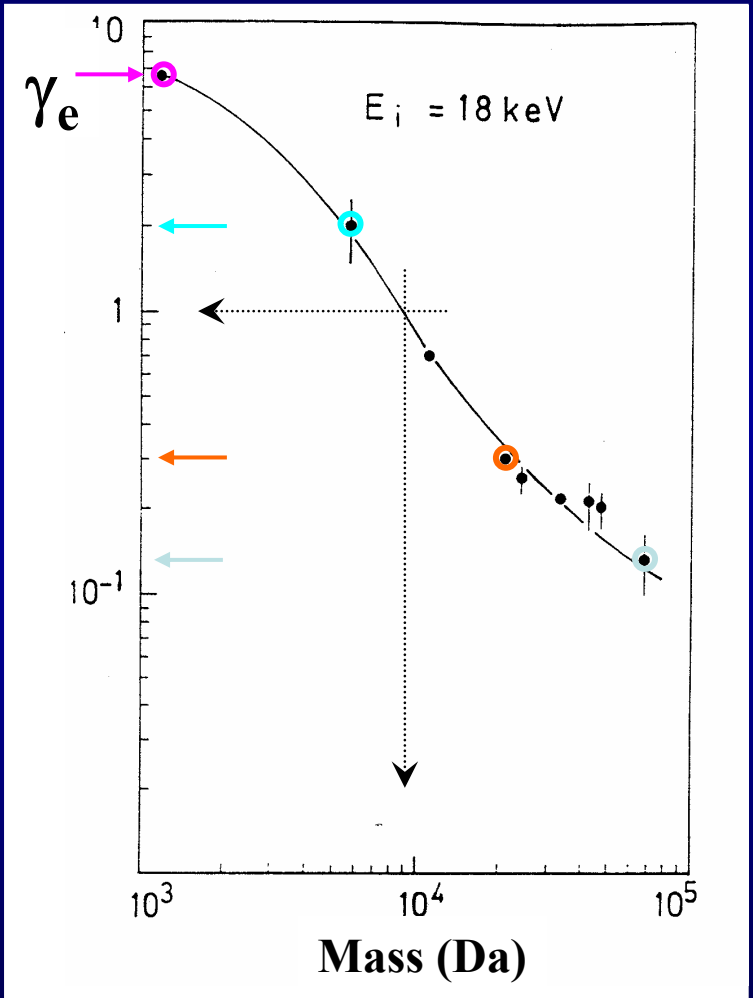
Nb ions > 10, (n1000)

(LD, MALDI…)

Transient Recorder

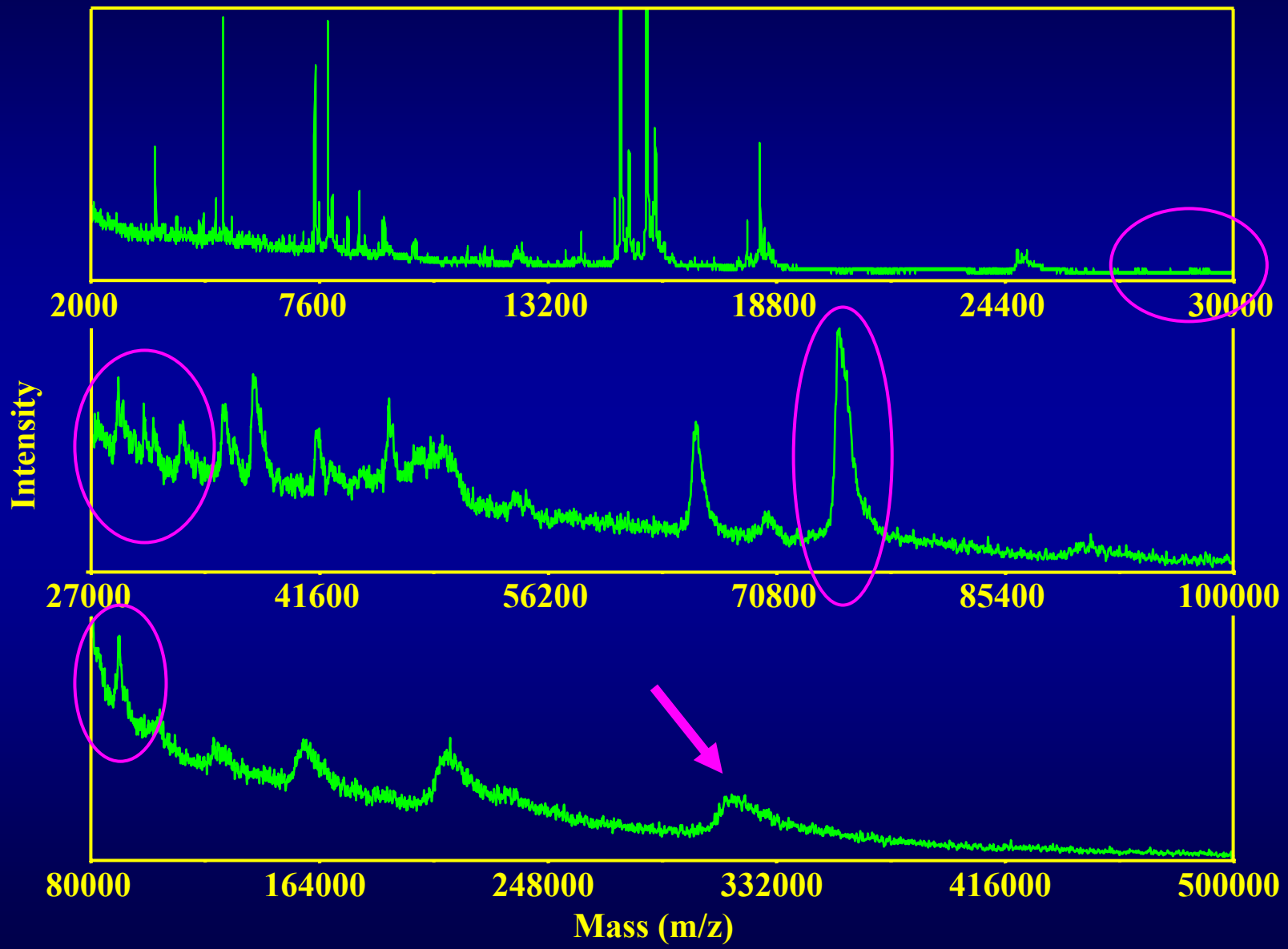
Gain / MCP ~ 10^{3-4}

Electronic emission from a CsI coated surface



$$\gamma_e \sim M^{0.6}\, f(V)^{(1\text{-}4)}$$

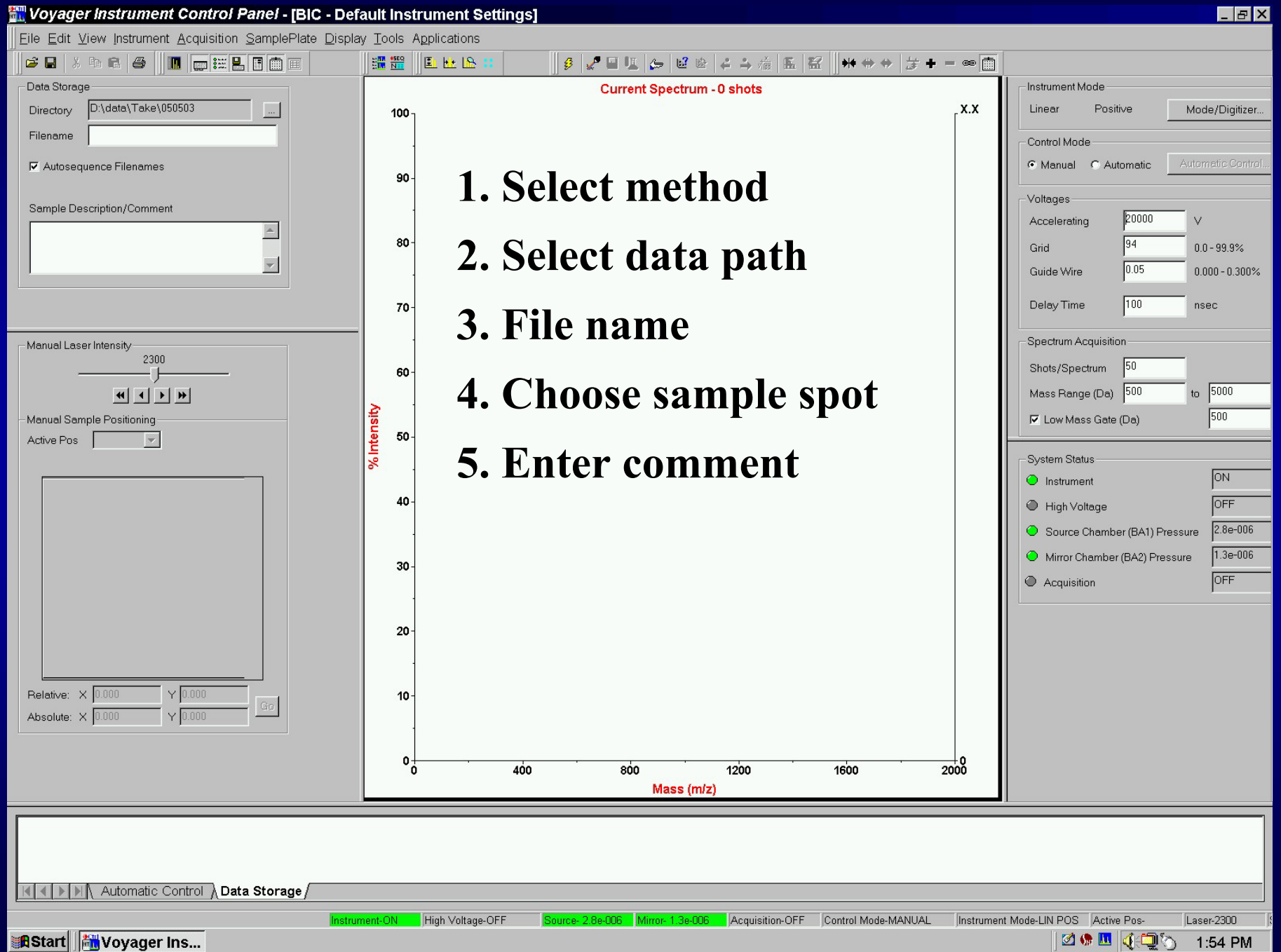
Protein extract from mouse anterior prostate



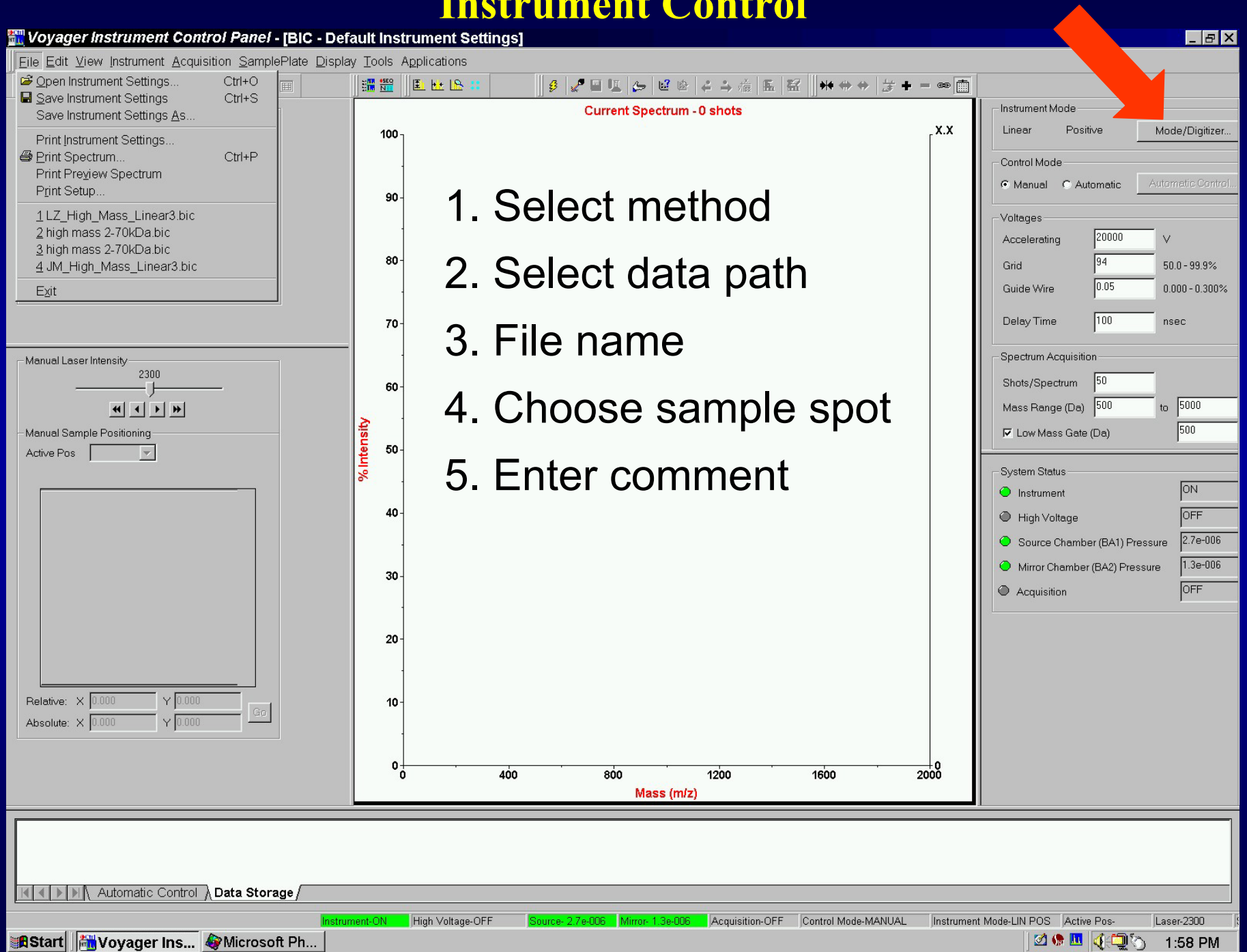
Signal Optimization

To Optimize…	Adjust…
Signal intensity	Laser intensity
Resolution	Delay Time Guide Wire Voltage% Grid Voltage%
Signal-to-Noise Ratio	Accelerating Voltage Guide Wire Voltage% Shots/Spectrum Low Mass Gate

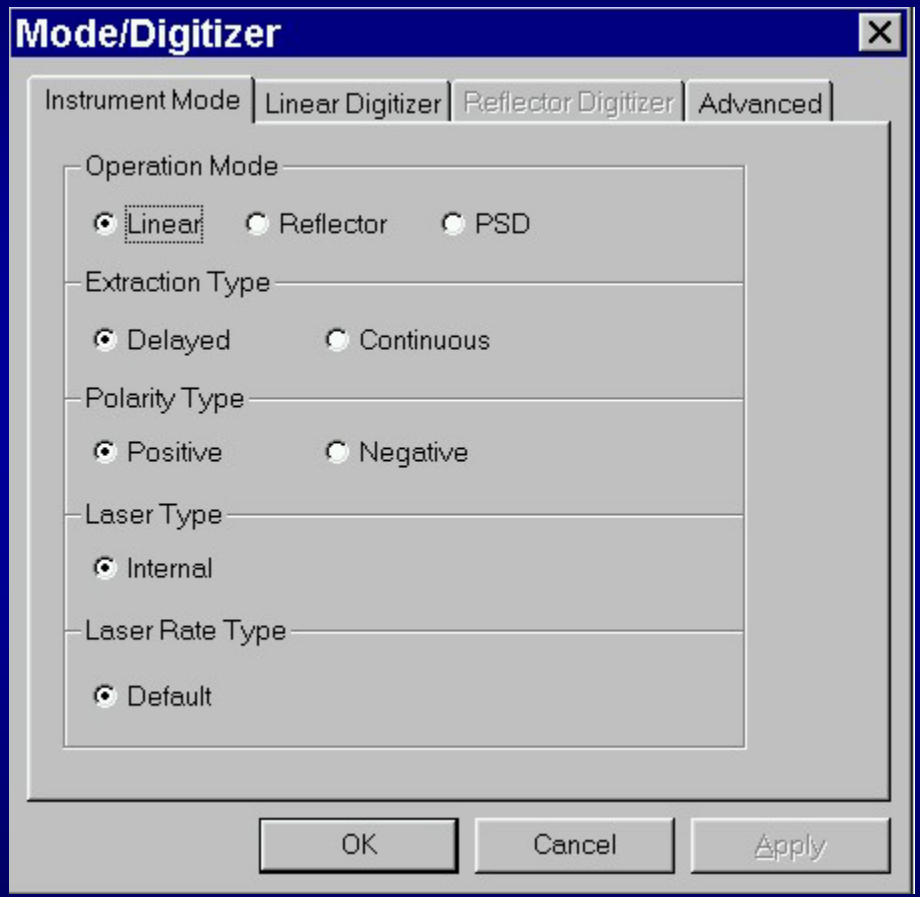
Instrument Control

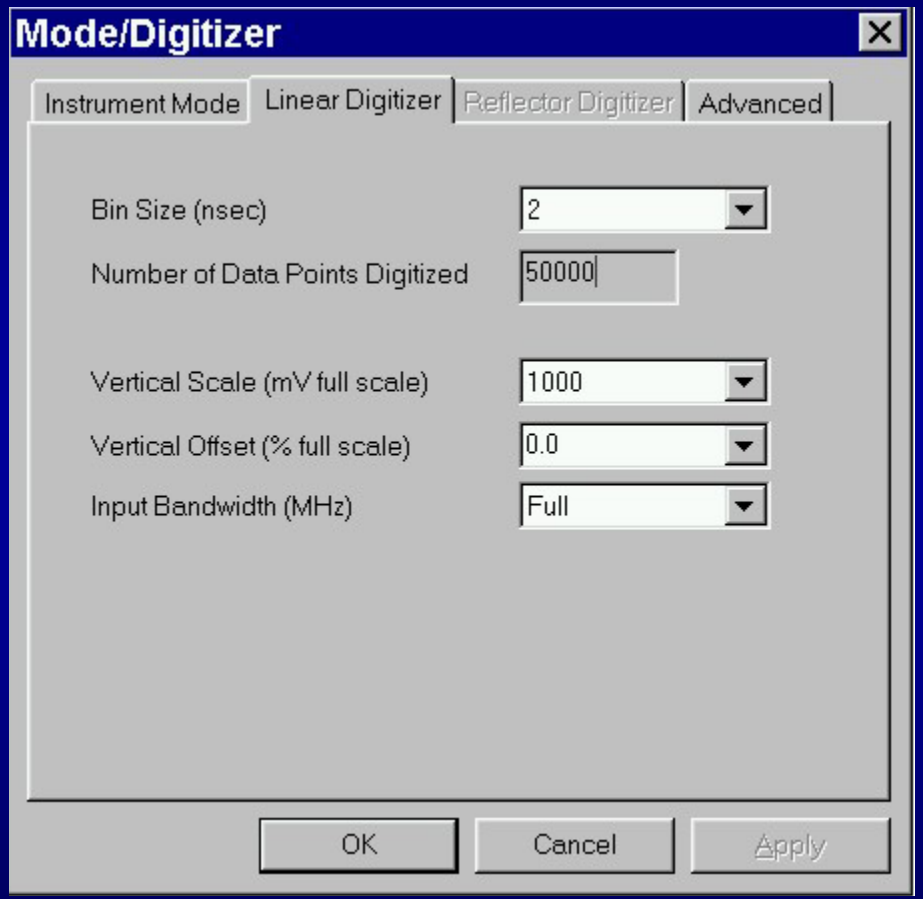


Instrument Control



Instrument Parameters

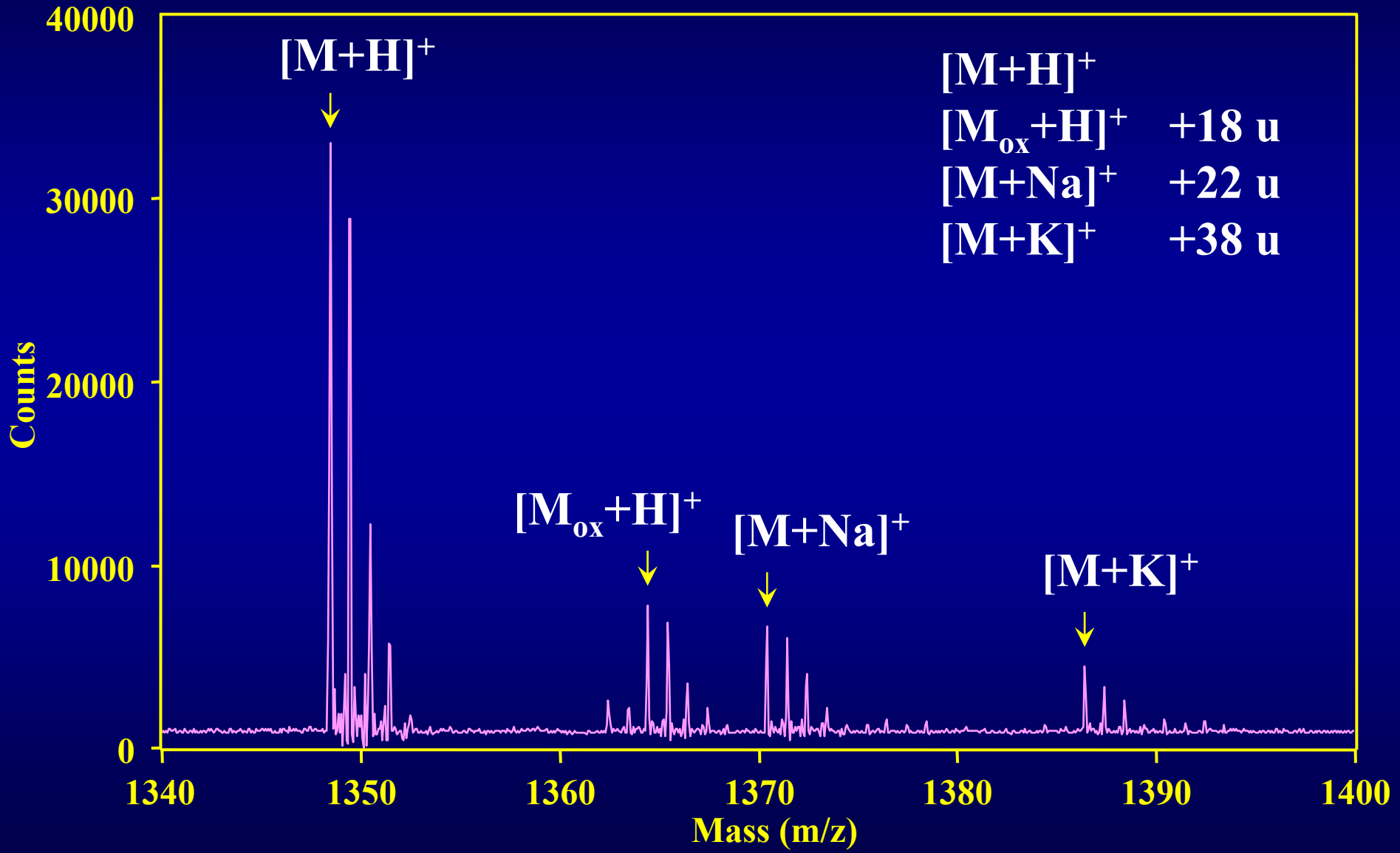




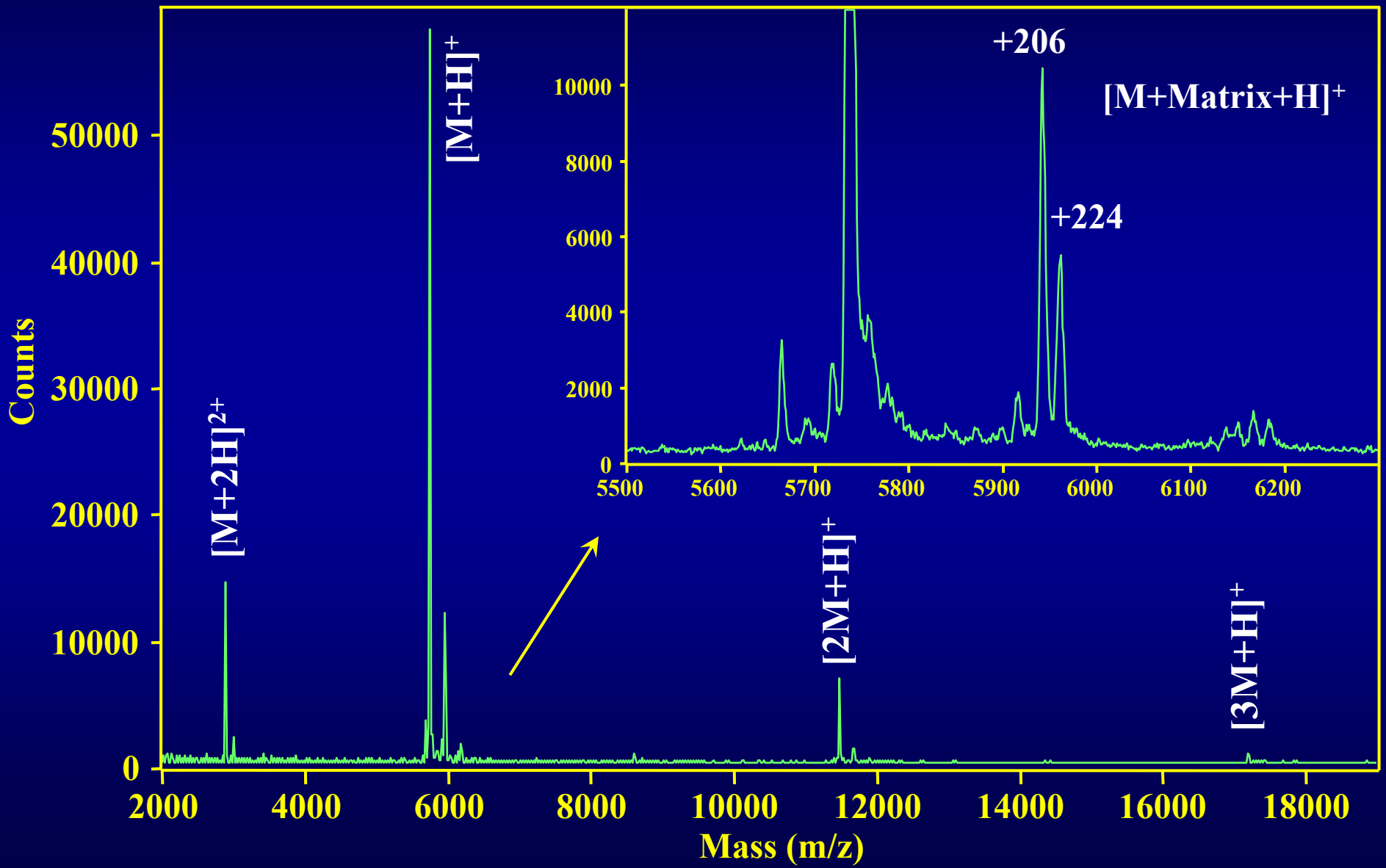
Instrument Parameters

Experiment	Bin size	Vertical scale
Peptides – linear mode	0.5 – 1 ns	200 mV – 1000 mV
Proteins – linear mode		
5 to 20 kDa,	4 – 10 ns	200 mV
> 20 kDa	10 – 20 ns	50 – 200 mV
Peptides – reflex mode	1 – 2 ns	50 – 200 mv

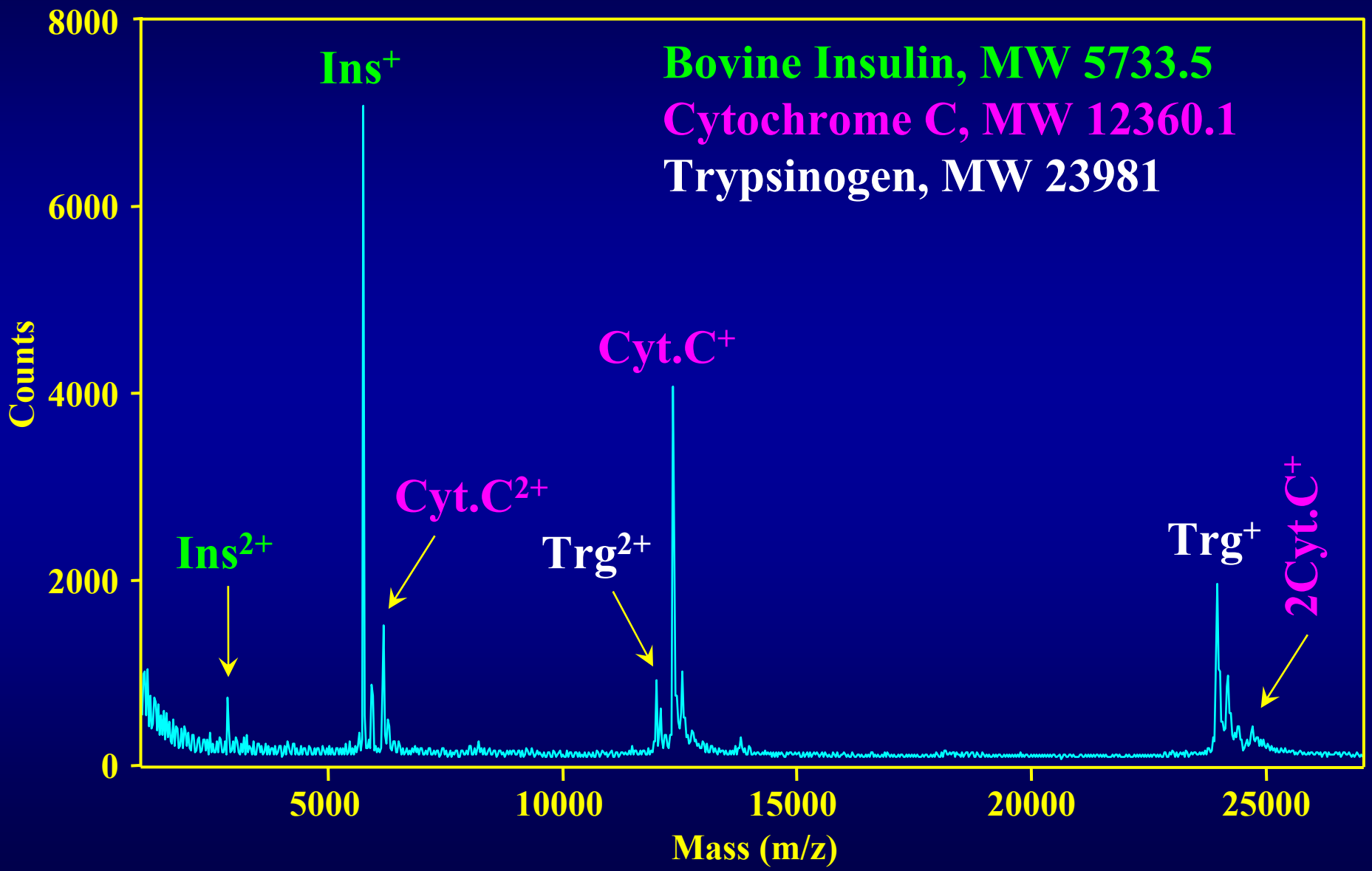
Substance P in HCCA



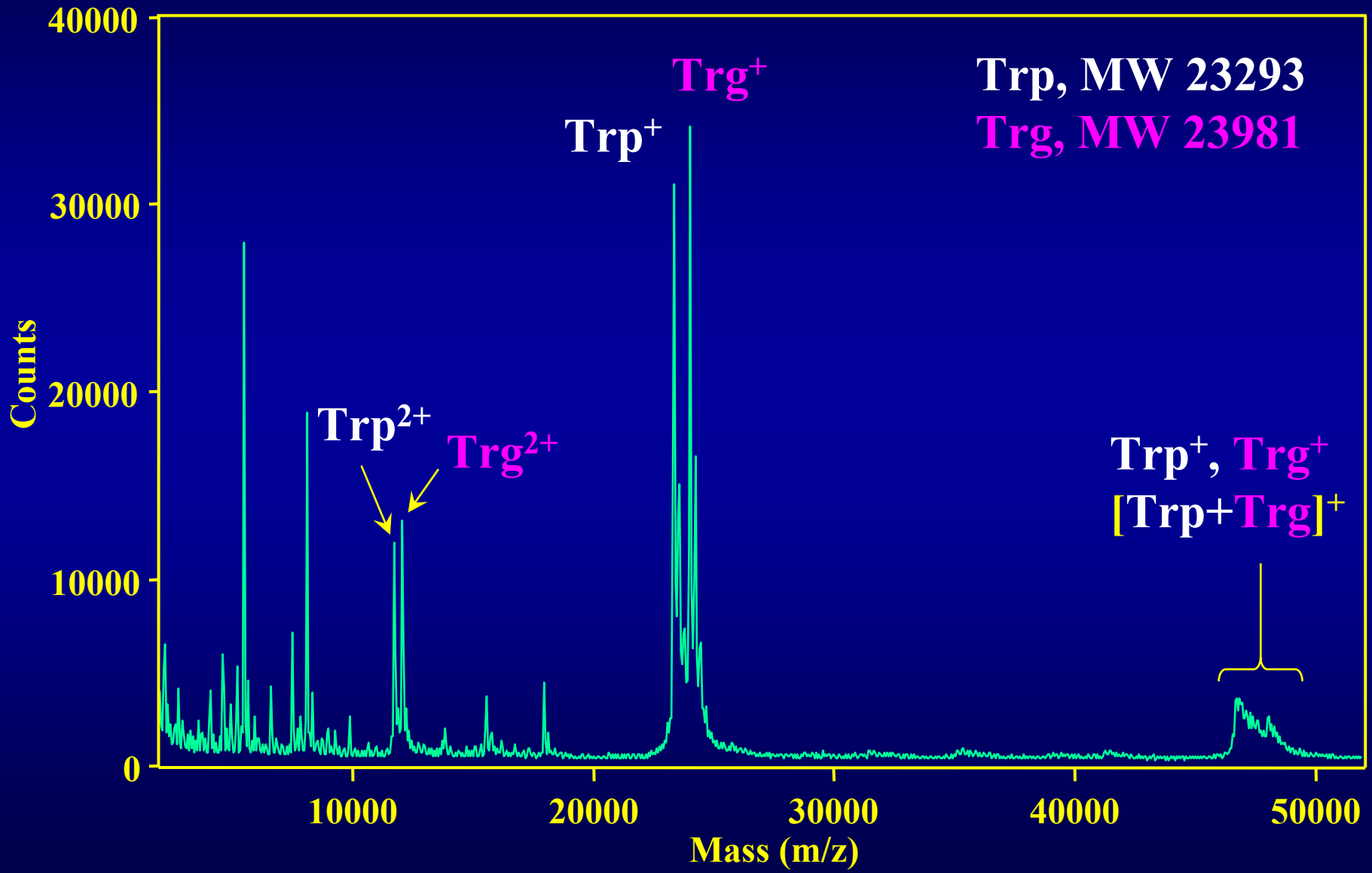
Bovine Insulin in Sinapinic Acid



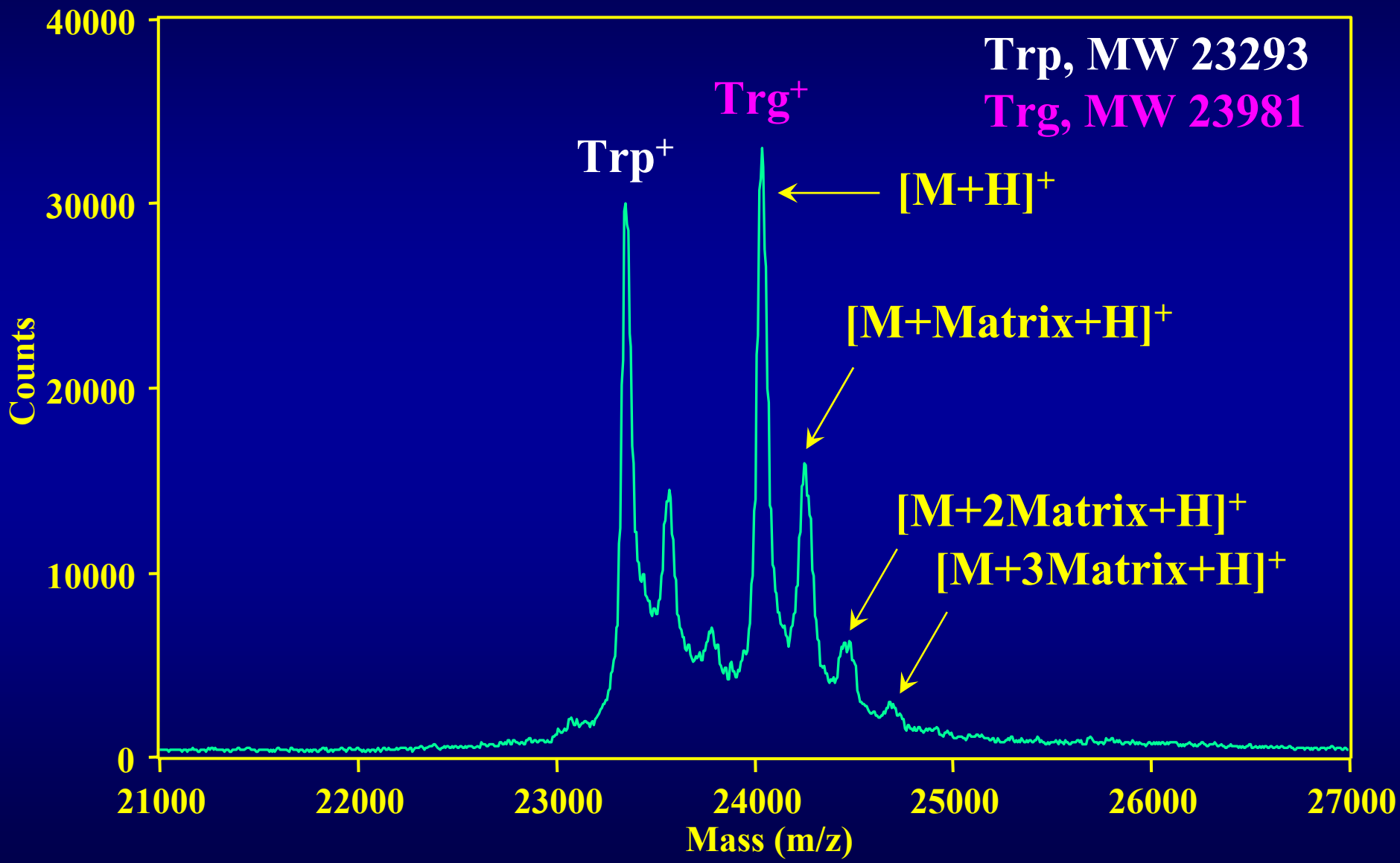
Mixture of 3 Proteins in SA



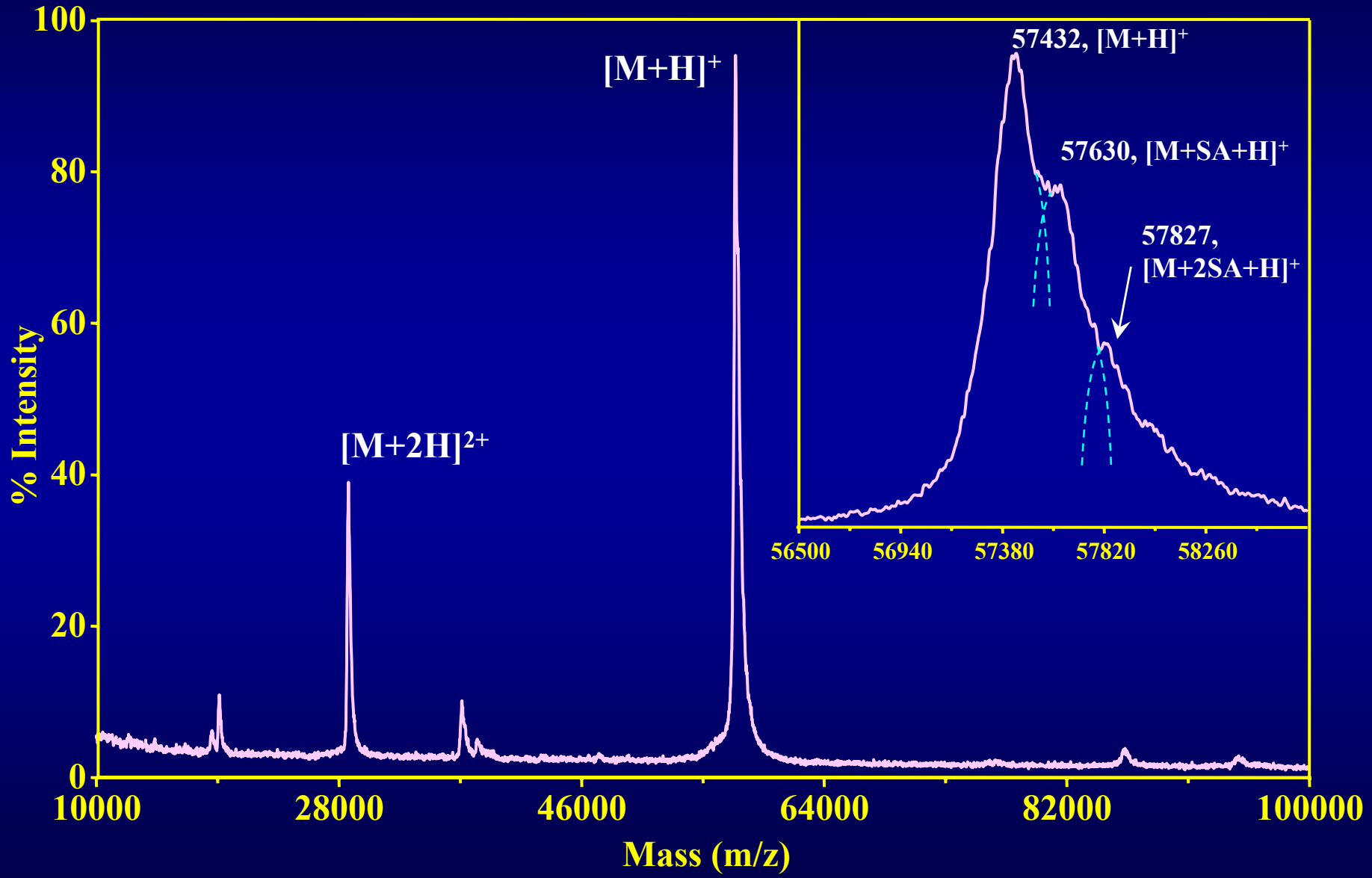
Mixture of Trypsin and Trypsinogen



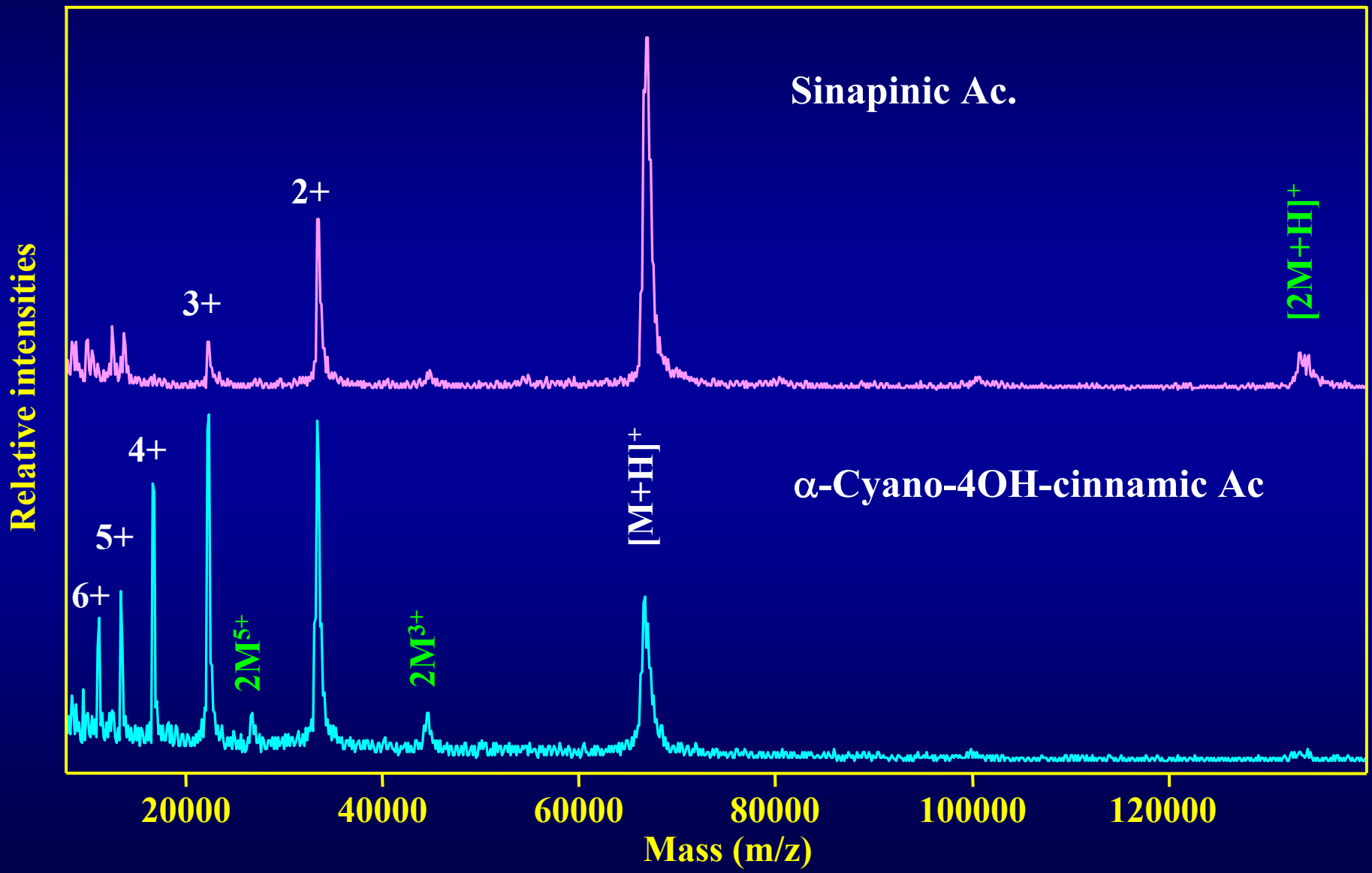
Mixture of Trypsin and Trypsinogen



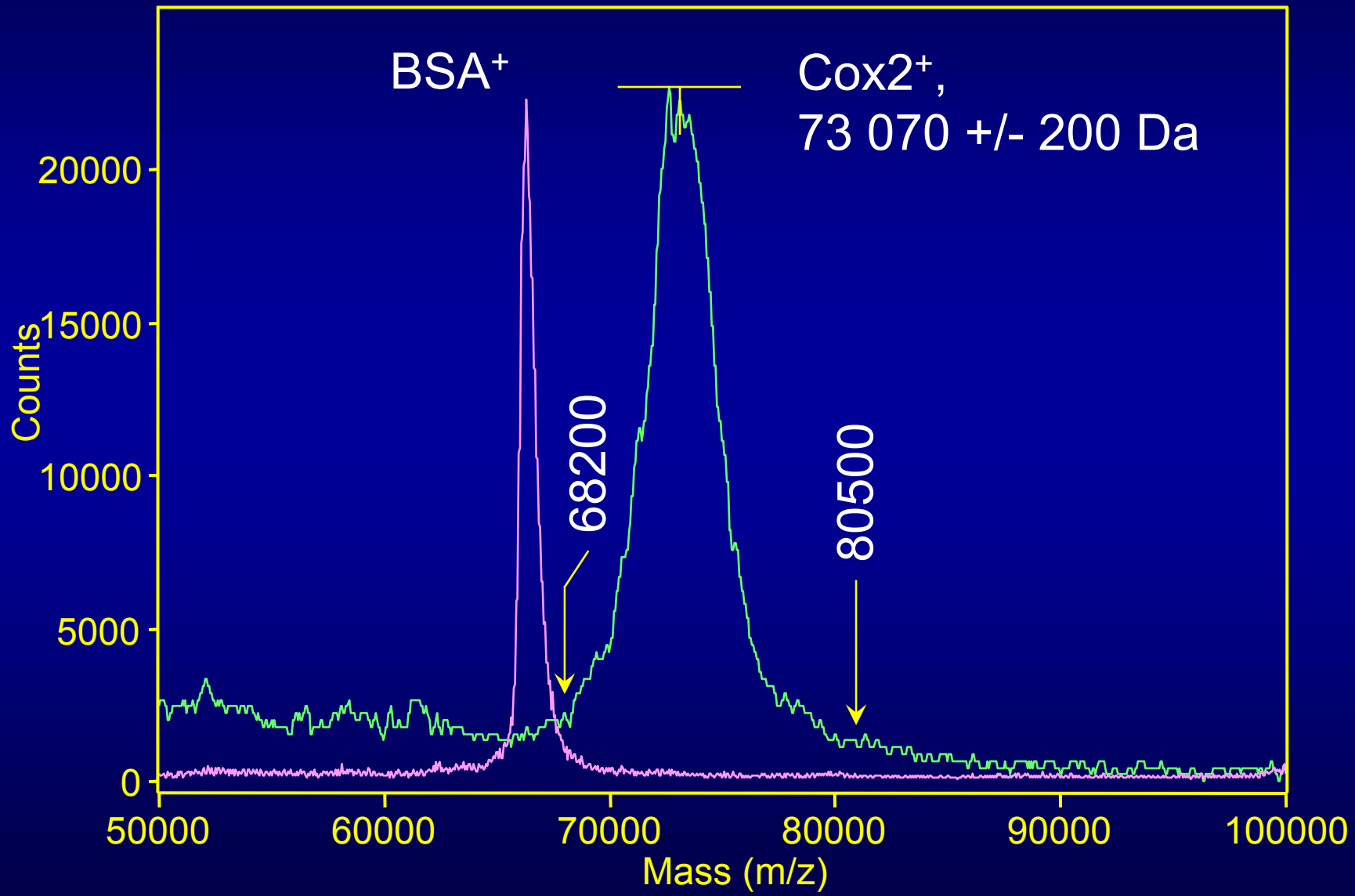
MALDI TOF MS of Glucose 6 Phosphate Dehydrogenase



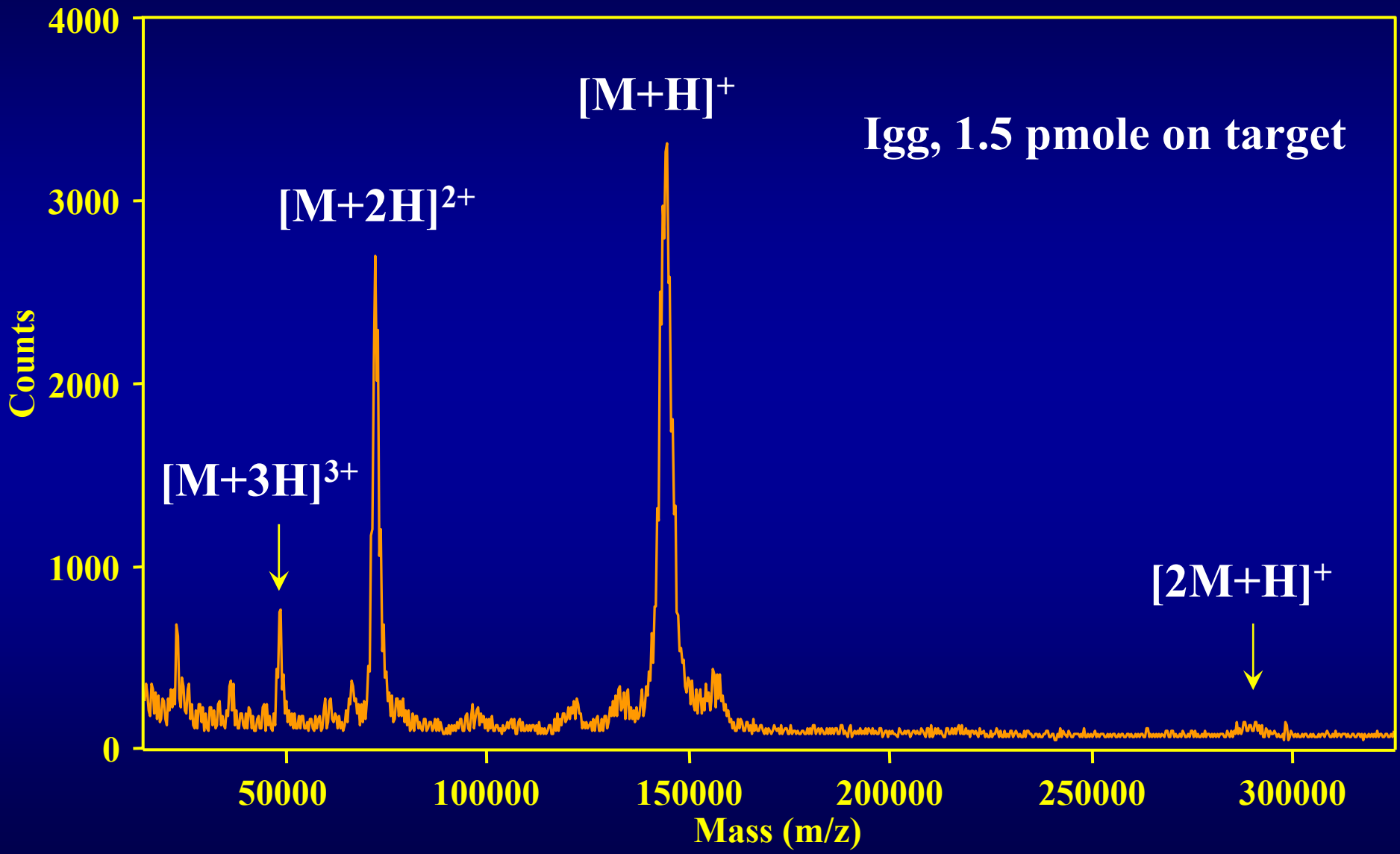
Influence of the Choice of Matrix



Cox2 in Sinapinic Acid (Glycoprotein)



IgG in Sinapinic Acid



MALDI-TOF Mass Spectrometry

Performances

- Biological materials : Peptides, Proteins, Polysaccharides, Polynucleotides...
- Mass accuracy : $1000 \leq \text{Mass} \leq 30{,}000$ Da $\Rightarrow \leq 10^{-4}$
  Mass $> 30{,}000$ Da $\Rightarrow \geq 10^{-4}$
- Sample amounts : Mass analysis $\Rightarrow$ a few fentomoles to a few picomoles.
  Structure analysis $\Rightarrow$ high femtomole range

Advantages

- Rapid, easy sample preparation,
- Large mass scan (Routine, up to 150,000 Da),
- Mixture analysis,
- Tolerance towards impurities (buffers, salts...).

Disadvantages

- No on-line coupling possibilities

Getting Started…

- Fill out form
- Check pressure gauges and instrument status
- Start Voyager software
- Load sample plate

Before you leave…

- Eject plate (and press ‘load no plate’)
- (Close Voyager software)
- Complete form
- Report problems with the instrument to a MSRC member