PerSeptive Biosystems
Voyager Elite

MALDI-TOF Mass Spectrometer

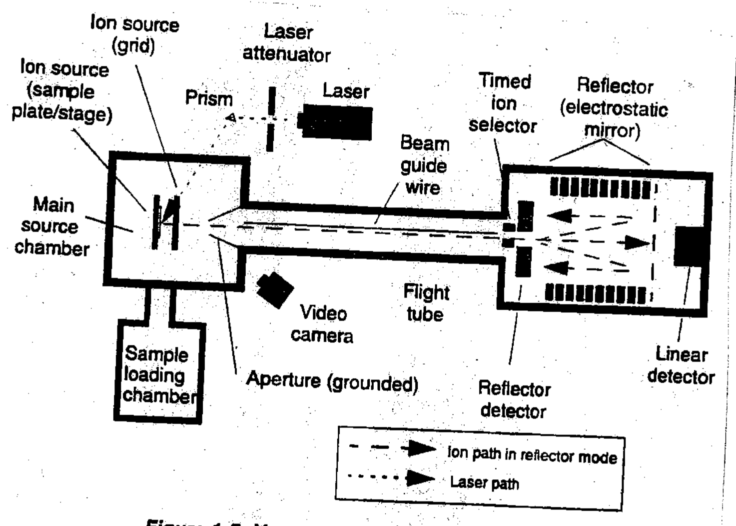

Figure 1-5 Voyager-Elite Mass Spectrometer

General Information file

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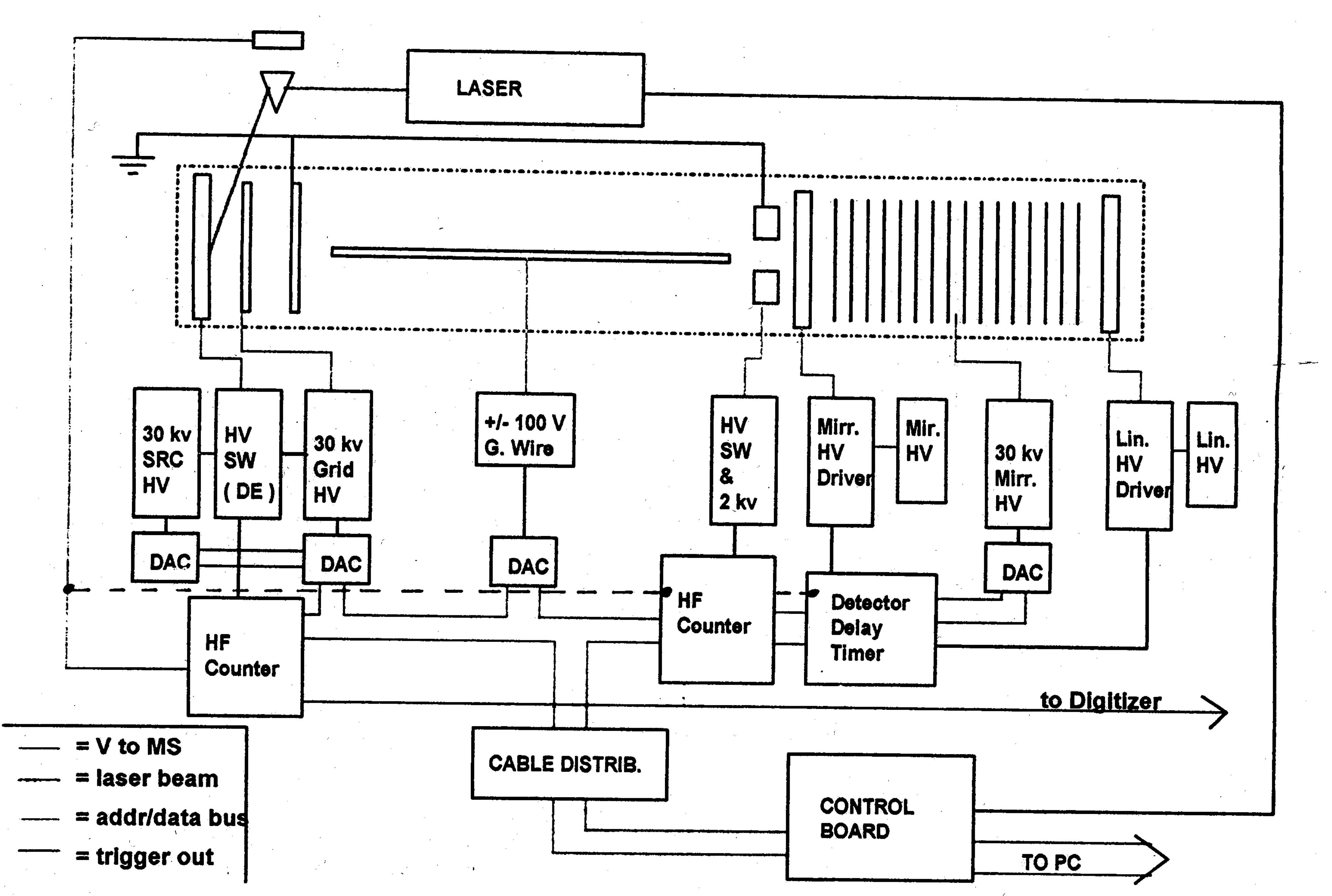
Parts of the system include:

- **Mass spectrometer**—A time-of-flight mass spectrometer, described in Section 1.3.2, Mass Spectrometer. The high-vacuum system of the mass spectrometer is described in Section 1.3.3, Vacuum System.
- **Computer/Data System**—A computer that operates the Voyager control software and the Voyager processing software. You control the mass spectrometer using the computer.
- **Digitizer**—An analog-to-digital converter that allows the signal from the mass spectrometer to be transferred to the computer.
- **Video Monitor**—A monitor that displays a real-time video image of the sample spot for examination during acquisition.
- **Control Stick**—A peripheral device that controls position of the sample plate in the mass spectrometer. Allows you to start and stop acquisition and transfer data to processing software.


LASER
30 kv SRC HV
HV SW (DE)
30 kv Grid HV
+/- 100 V G. Wire
HV SW & 2 kv
Mirr. HV Driver
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30 kv Mirr. HV
Lin. HV Driver
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DAC
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HF Counter
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Detector Delay Timer
to Digitizer
CABLE DISTRIB.
CONTROL BOARD
TO PC
= V to MS
= laser beam
= addr/data bus
= trigger out

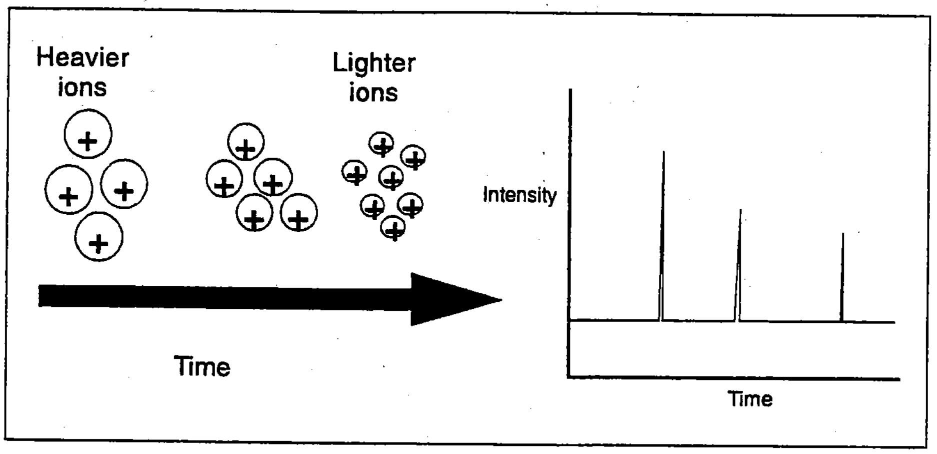

Figure 1-3 Time of Flight Analysis

The time required for ions to reach the detector at the opposite end of the flight tube is measured. The number of ions reaching the detector at any given time is also measured, and is referred to as ion intensity (abundance) or signal intensity.

Drift time is proportional to the square root of the mass and is calculated by the following equation:

$$t = s\left(\frac{m}{(2KE)}\right)^{1/2}$$

$$E_c = \frac{1}{2} m v^2 = \frac{1}{2} m \left(\frac{s}{t}\right)^2 \Rightarrow t = s\left(\frac{m}{2E_c}\right)^{1/2}$$

where:

t	=	drift time
s	=	drift distance
m	=	mass
KE	=	kinetic energy

Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Analysis of Peptides Using Post-Source Decay

A.M. Falick, PerSeptive Biosystems Western Technical Center, San Francisco, CA

In recent years, mass spectrometry has become an essential tool for the analysis of biopolymers (1-3). Accurate molecular weight information can be determined in a few minutes on extremely small quantities of material in a straightforward fashion. In addition, it is also possible to obtain sequence information using various mass spectrometric methods such as MS/MS (mass spectrometry/mass spectrometry). This Technical Bulletin describes the use of post-source decay for peptide analysis.

MS/MS Analysis

MS/MS analysis requires the use of two stages of mass separation. A four-sector mass spectrometer consisting of two double-focusing magnetic sector instruments placed end-to-end is frequently used for this type of analysis (4,5). The first instrument is used to select the ions to be analyzed; in the case of a peptide mixture, ions formed from a selected peptide are allowed to pass into the second mass spectrometer, while ions of the "wrong" mass are rejected. A collision cell filled with a low pressure of an inert gas such as helium is placed between the two mass spectrometers. Selected ions pass into the cell and collide with gas molecules; as a result some ions decompose due to the internal energy gained in the collision. The fragments that are formed are mass-analyzed in the second mass spectrometer. The resulting mass spectrum, often called a collision-induced dissociation (CID) spectrum, yields a great deal of information about the starting compound.

MS/MS analysis can also be performed using quadrupole instruments referred to as triple quadrupoles (6). The first quadrupole selects the ion of interest, the second is a collision cell, and the third is used to analyze the fragments. Quadrupoles generally operate

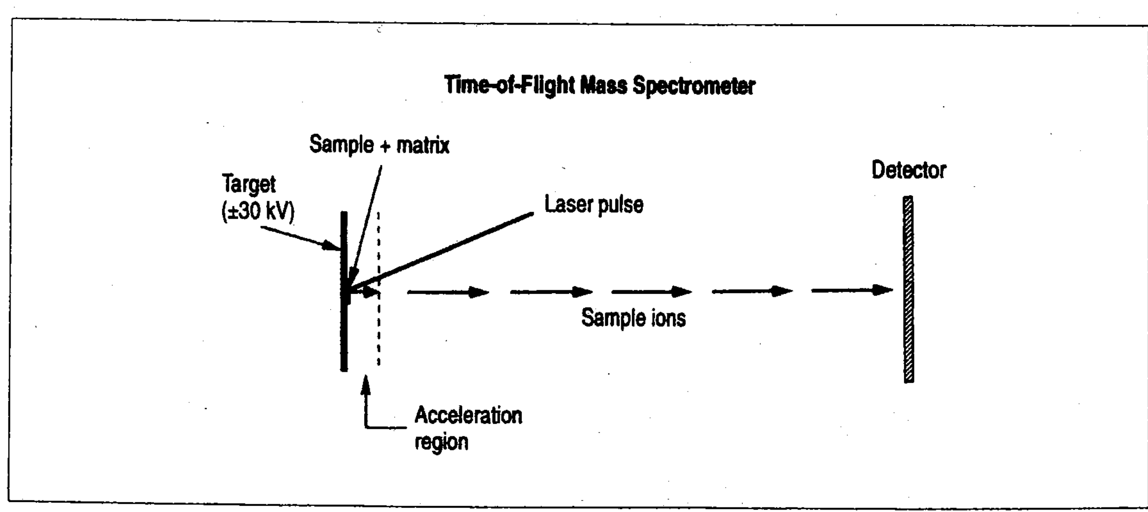

Figure 1. *Principle of a linear TOF mass spectrometer. Ions are formed at the target by a laser pulse striking a mixture of sample and matrix placed on the sample plate (target).*

at lower energies than sector instruments and while the fragmentation is often simpler, it may be less informative.

Post-Source Decay (PSD) Analysis

The post-source decay method (7) used in time-of-flight (TOF) mass spectrometers has much in common with MS/MS in that fragments of a selected analyte molecule are formed and analyzed in the instrument. This method has been successfully used to determine and verify peptide sequences (8,9); the spectra obtained are very similar to those obtained using the double mass spectrometer techniques (10).

In traditional mass spectrometric analysis, analyte ions are normally produced by matrix-assisted laser desorption ionization (MALDI) (11, 12) yielding very high sensitivity results. The basic TOF instrument (without PSD) operates as shown in Figure 1. Ions are formed in the ion source by a laser pulse, then rapidly accelerated to a high kinetic energy (20-30 keV). In a linear TOF instrument, once ions leave the short acceleration region, they travel down a 1-2 meter tube to the detector, where their arrival is timed. Mass-to-charge ratio, m/z, is determined from the time elapsed from ion formation (laser pulse) to ion arrival time at the detector. Ions typically acquire enough internal energy at the time of ionization to undergo unimolecular decomposition as they travel along the flight tube; in a linear instrument, however, both intact ions and any fragment ions and neutrals arrive at the detector at virtually the same time. As a result, only one peak is detected, at a time corresponding to the precursor ion mass.

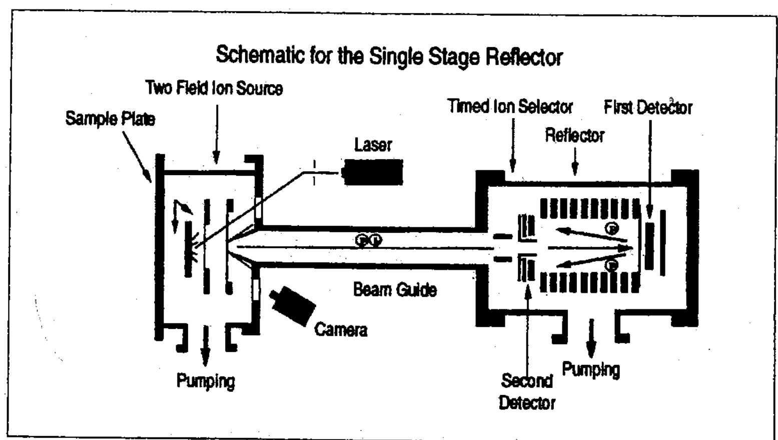

Figure 2. Diagram of a reflecting type TOF mass spectrometer. This instrument is equipped with a beam guide for more efficient ion transmission and a timed ion selector.

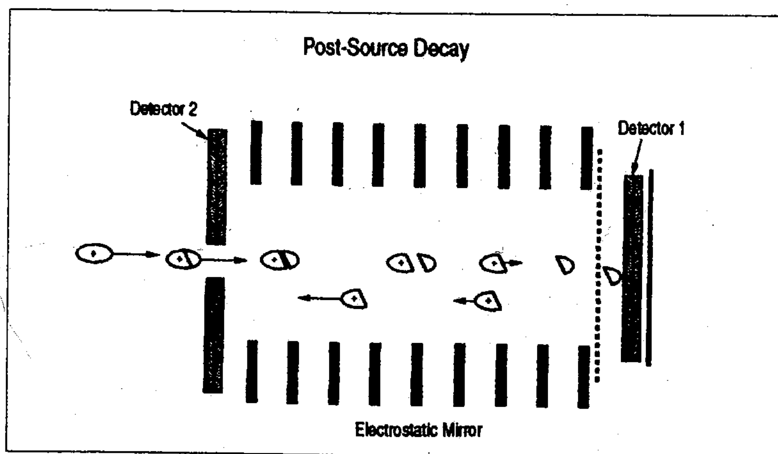

Figure 3. Electrostatic mirror (reflector) portion of a TOF instrument. An ion fragments before entering the mirror (anywhere in the field free region) and the charged and neutral fragments enter the mirror together. The neutral fragment strikes the detector at the back of the mirror (normally turned off in this mode) and is lost. The charged fragment turns around in the mirror and is detected at the detector located at the front of the mirror.

Some TOF instruments are equipped with an electrostatic mirror (reflector) (13) that reflects ions back towards a second detector (Figure 2). The original function of the reflector is to reduce the translational kinetic energy spread of the ions so as to improve resolution. Faster ions penetrate farther into the reflecting field and thus travel a longer distance than slower ions. As a result, if instrument parameters are set properly, all ions of the same m/z arrive at the detector at nearly the same time.

It is important to realize that all ions formed in the source and accelerated normally will have approximately the same amount of translational kinetic energy

and thus penetrate about the same distance into the mirror. Ion speeds however, differ according to their m/z values and therefore, so do their arrival times. This includes fragment ions formed concurrent with or immediately after ionization ("prompt" fragments).

Fragment ions formed as a result of unimolecular decay after acceleration of the precursor ion out of the ion source and before reflection (post-source decay), are quite different. These ions do not follow the same path as fully accelerated ions and as a result, reach the reflector detector at a different time than either their intact precursor ions or normal fragment ions formed in the source. Neutral fragments are not reflected and are not detected at the reflector detector (Figure 3).

Consider the example of a singly charged ion of mass 1000 that decomposes to an m/z 500 ion in flight after it leaves the ion source. The precursor ion (m/z 1000) will reach the reflector detector at the expected time for its mass; the fragment ion will have less kinetic energy (half as much in this example) and will be moving more slowly and thus not penetrate as far into the mirror as would a fully accelerated ion of the same mass. While these properties (slower speed, shorter path) have opposite effects on the arrival time, the result is that the ion arrives at the detector at a slightly shorter time than a normal fragment of the same mass. A fragment of m/z 250 from the same precursor would have even less kinetic energy and would penetrate the mirror even less than the m/z 500 ion. However, given the instrument parameters, one can accurately calculate the mass of a fragment ion formed in flight from its arrival time. The resulting spectrum contains peaks at the masses of fragments formed by PSD.

Ions that do not penetrate fully into the mirror are not refocused as effectively as ions that travel almost the entire mirror length. Ions that have less kinetic energy than this optimum value can still be correctly focused simply by making the reflecting field weaker. The kinetic energies of PSD fragment ions vary according to their masses, and as a result, no one reflector field will be optimal for all ions. The range of energies that can be properly focused is such that if a mirror voltage is varied in 5-10 steps, all of the ions of interest can be satisfactorily focused and detected. Reducing the mirror voltage has an additional advantage: Fully accelerated ions are not reflected (they pass through the back of the mirror) and as a result, do not appear in the spectrum; this includes prompt fragment ions formed in the source and non-fragmenting ions from other species that may be present. The PSD spectrum is formed by assembly of the 5-10 steps, normally accomplished by the data system of the mass spectrometer.

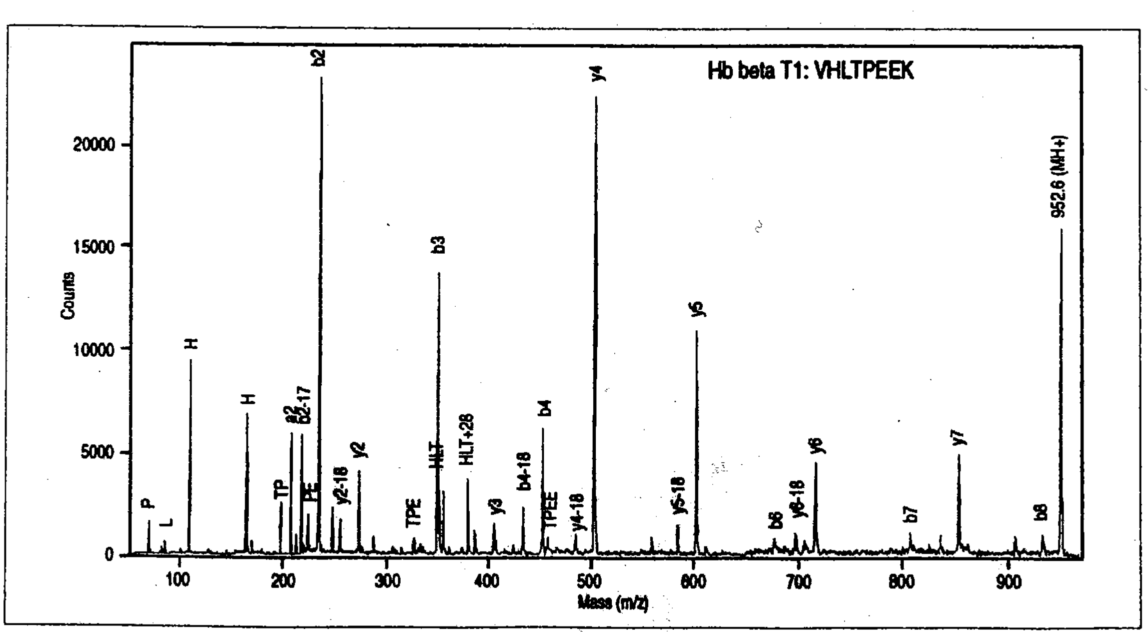

Figure 4. PSD spectrum of ~2 picomoles of the hemoglobin tryptic peptide VHLTPEEK. Peaks in the spectrum are marked in accordance with the standard nomenclature for fragment ions (14).

A TOF instrument that is properly equipped to do PSD analysis usually includes a timed ion selector (Figure 2). This device, located along the flight path of the precursor ions, allows the selection of precursor ions of a certain mass so that the PSD spectrum contains only fragments from the selected precursor ion. As a result, a pure PSD spectrum of one component of a mixture can be obtained without having to perform a prior separation. The timed ion selector works simply by opening an electrostatic gate at the arrival time of the desired precursor ion. These ions pass through normally; ions arriving at different times (different m/z values), however, are not allowed to pass. Typically, the gate can be set to discriminate against ions that differ from the desired ion by approximately one percent or more.

Peptides typically fragment to give both sequence ions and amino acid specific ions at low masses. Figure 4 shows the PSD spectrum of a hemoglobin tryptic peptide (beta T1). The spectrum shows a complete series of y ions (cleavage between the carbonyl and amino groups in the peptide backbone with charge retention on the C-terminal fragment). From differences in mass between the members of this series of ions, one can determine or verify the amino acid sequence of the peptide. The low mass ions marked with single letter codes are characteristic of the presence of specific amino acid residues in the peptide and provide information as to the amino acid composition of the peptide.

References

1. Biemann, K. 1992. Annual Review of Biochemistry **61**:977-1010.
2. Burlingame, A.L., R.K. Boyd, S.J. Gaskell. 1994. Analytical Chemistry **66**:634R-683R.
3. Chiat, B.T., S. Kent. 1992. Science **257**:1885-1894.
4. Walls, F.C., M.A. Baldwin, A.M. Falick, B.W. Gibson, S. Kaur, D.A. Maltby, B.L. Gillece-Castro, K.F. Medzihradszky, S. Evans, A.L. Burlingame. 1990. in Biological Mass Spectrometry. Ed., Burlingame, A.L., J.A. McClosky (Elsevier, Amsterdam) pp. 129-146
5. Ashcroft, A.E., P.J. Derrick. 1991. in Four-sector tandem mass spectrometry of peptides. Ed., Desiderio, D.M. (CRC Press, Boca Raton FL) pp. 121-138.
6. Yost, R.A., R.K. Boyd. 1990. Meth. Enzymology **193**:154-200.
7. Spengler, B., D. Kirsch, R. Kaufmann, E. Jaeger. 1992. Rapid Communications in Mass Spectrometry **6**:105-108.
8. Kaufmann, R., B. Spengler, R. Lutzenkirchen. 1993. Rapid Communications in Mass Spectrometry **7**:902-910.
9. Kaufmann, R., D. Kirsch, B. Spengler. 1994. Int. J. Mass Spectrom. Ion Processes **131**:355-385.
10. Rouse, J.C., W. Yu, S. Martin. 1995. J. Am. Soc. Mass Spectrometry **6**:822-835.
11. Karas, M., D. Bachmann, U. Bahr, F. Hillenkamp. 1987. Int. J. Mass Spectrom. Ion Processes **78**:53-68.
12. Hillenkamp, F., M. Karas, R. Beavis, B.T. Chait. 1991. Analytical Chemistry **63**:1193A-1203A.
13. Mamyrin, B.A., V.I. Karataev, D.V. Schmikk, V.A. Zagulin. 1973. Sov. Phys. JETP **37**:45.
14. Biemann, K. 1990. Meth. Enzymology **193**:886-887.

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Matrix	Application	Structure	Color of crystals/ solution	Matrix Solution
Sinapinic acid (see mass spectrum on page C-2) MW 224.07	• Peptides • Proteins	CH══CHCOOH CH_3O OCH$_3$ OH PB100251	White	• 10 g/liter in 50:50 water/ethanol[1] • 10 g/liter in 70:30 water/acetonitrile (0.1% TFA)[2]
Alpha-cyano-4-hydroxy cinnamic acid (αCHCA) (see mass spectrum on page C-2) MW 189.04	• Peptides • Proteins	CH══C(CN)COOH OH PB100252	Yellow	• 10 g/liter in 70:30 water/acetonitrile (0.1% TFA)[3] • 10 g/liter in 50:50 water/acetonitrile (0.1% TFA)
2,5-dihydroxybenzoic acid (2,5-DHB) MW 154.03	• Peptides (mixtures) • Carbohydrates • Glycolipids (neg ion mode) • Polar synthetic polymers	COOH OH HO PB100253	White	10 g/liter in 90:10 water/ethanol[4]

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Matrix	Application	Structure	Color of crystals/ solution	Matrix Solution
2-(4-hydroxy-phenylazo)-benzoic acid (HABA) MW 242.07	• Proteins • Polar and nonpolar synthetic polymers	COOH, N=N, OH PB100255	Orange	~1.3 g/liter in 50:50 water/acetonitrile or in 40:40:20 water/ acetonitrile/methanol[5]
2-aminobenzoic (anthranilic) acid MW 137.05	Oligonucleotides	COOH, NH_2 PB100256	Yellow	~10 g/liter in 80:20 water/acetonitrile (20% w/w nicotinic acid can be added)[6]
3-hydroxypicolinic acid MW 139.03	Oligonucleotides	COOH, OH, N PB100257	Light brown	~80 g/liter in 50:50 water/acetonitrile[7]
Dithranol MW 226.06	Nonpolar synthetic polymers + Polar polymers.	OH, O, OH PB100258	Yellow	10 g/liter in tetrahydrofuran[8]

Matrix	Application	Structure	Color of crystals/ solution	Matrix Solution
2,4,6 Trihydroxy acetophenone (THAP) MW 168.04	Oligonucleotides	$COCH_3$ HO OH OH PB100261	White	0.5 M 2,4,6-THAP in ethanol, mix with 0.1 M aqueous diammonium hydrogen citrate (2:1)[9]
Picolinic acid (YAG laser only, 266 nm) MW 123.03	Oligonucleotides	COOH N PB100260	White	1.2 to 2.4 M (150 g/liter to 300 g/liter) in water[10]
Nicotinic acid (YAG laser only, 266 nm) MW 123.03	Proteins	COOH N PB100254	White	0.05M in water[11]

1. Beavis, R.C., B.T. Chait, *Rapid Commun. Mass Spectrom.* 1989, **3**, 432-435
2. Beavis, R.C., B.T. Chait, *Anal.Chem.* 1990, **60**, 1836-1840
3. Beavis, R.C., B.T. Chait, *Org. Mass Spectrom.* 1992, **27**, 156-158
4. Strupat, K., M. Karas, F. Hillenkamp, *Int. J. Mass Spectrom. Ion Proc.* 1991, **111**, 89-102
5. Juhasz, P., C.E. Costello, K. Biemann *J. Am. Soc. Mass Spectrom.* 1993, **4**, 399-409
6. Nordhoff, E. *et al., Rapid Commun. Mass Spectrom.* 1992, **6**, 771-776
7. Wu, K.J., A. Steding, C.H. Becker *Rapid Commun. Mass Spectrom.* 1993, **7**, 142-146
8. Juhasz, P., C.E. Costello *Rapid Commun. Mass Spectrom.* 1993, **7**, 343-351
9. Schar, M., *et al., Nucleic Acids Research* 1993, **21**, **14**, 3191-3196
10. Chen, C.H., *et al., Rapid Commun. Mass Spectrom.* 1994, **8**, 673-677
11. Karas, M., U. Bahr, F. Hillenkamp Int. *J. Mass Spectrom. Ion Proc.* 1989, **92**, 231-242

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To: mbier@andrew.cmu.edu
Message-ID: <970729102218.2260928c@CPWSCA.PSC.EDU>

Hi Mark,
This came in that I thought would be of interest - you're not on the ABRF network anymore are you?

Otherwise, I do want to come over for some ESMS, but I need to get a peptide digest separated first. More on that soon.............JH

~At 9:39 AM -0800 7/28/97, Richard S. Johnson wrote:

>Dear ABRF:
> Does anyone have a favored sample preparation method for obtaining high
>sensitivity peptide measurements on a maldi-tof?

Richard,
I've had good luck using a modified version of Xiang and Beavis' "polycrystalline" method. It seem to work best with the stipled or roughened sample wells. I simply make a 50 mg/ml solution of alphacyano in 99% or 100% acetone and spot 0.1-0.3 ul to the position. After drying (almost instantaneous) I use a piece of hard paper to crush the crystals (or just wipe with a kimwipe, personal commun. with Ron Beavis,) and then wipe as hard as I can with a folded dry kimwiper. Then I make a 1:10 dilution of the aqueous sample in saturated alphacyano in 1:2 0.1%TFA/ACN, and spot 1.0 ul. As soon as the layer appears, remove the liquid, dry, and wash with 2 X 5ul deionized water. For the highest sensitivity, I've found you can get away spotting 1.0 ul of the saturated matrix and adding no more than 0.1-0.2 ul of the sample directly to the slide. If all goes well, I generally see high resolution but like you, greatly reduced numbers of good shots per sample, i.e. 1-4 shots before the signal degrades. So, this method might not be so hot for PSD or PSD/CAD. However, with this technique and acetylated angiotensinogen, I have been able to obtain better than 10:1 S/N with <800 attomoles spotting 50-100 nanoliters.
best of luck,

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