MALDI-TOF MS

Matrix-Assisted Laser Desorption/Ionization time-of-flight, Mass Spectrometry (MALDI-TOF MS)
(Matriks ile desteklenmiş lazer desorpsiyon/iyonizasyon uçuş zamanı kütle spektrometresi)

Dr. Meltem Yalınay Çırak

Gazi Üniversitesi Tıp Fakültesi
Tıbbi Mikrobiyoloji Anabilim Dalı

XXXIV. Mikrobiyoloji Kongresi
07-11 Kasım 2010

Belli bir zamanda, belli bir hücrede, belli bir dokuda, belli bir organizmada bulunan gereken tüm biyokimyasal tepkimeleri yürüten proteinlerin tamamı **proteom**

Proteomik ise belli bir organizmada bulunan proteinlerin toplamını incelemek için kullanılan yöntemlerin tümü

Proteomik çalışmalarda kullanılan yöntemler

- ELISA
- İki yönlü elektroforez (2D-PAGE)
- Doku ve protein çip teknolojisi (mikroarray)
- Kütle spektrometresi
  - MALDI-TOF MS (Matrix-assisted laser desorption/ionization time of flight Mass Spectrometry)
  - SELDI-TOF MS (Surface enhanced laser desorption/ionization time-of-flight Mass Spectrometry)

- Kantitatif kütle spektrometresi
- Lazer yakalama mikrodisseksiyon [Laser capture microdissection (LCM)]
- Protein-protein, protein-DNA bağlanma reaksiyonları için afinite kromatografi, floresans rezonans enerji transferi (FRET)
- Peptid ve proteinlerin üç boyutlu incelenmesi
  - X- ışını kristalografi, nüklear manyetik rezonans

Matrix-Assisted Laser Desorption/Ionization time-of-flight, Mass Spectrometry (MALDI-TOF MS)
(Matriks ile desteklenmiş lazer desorpsiyon/iyonizasyon uçuş zamanı kütle spektrometresi)

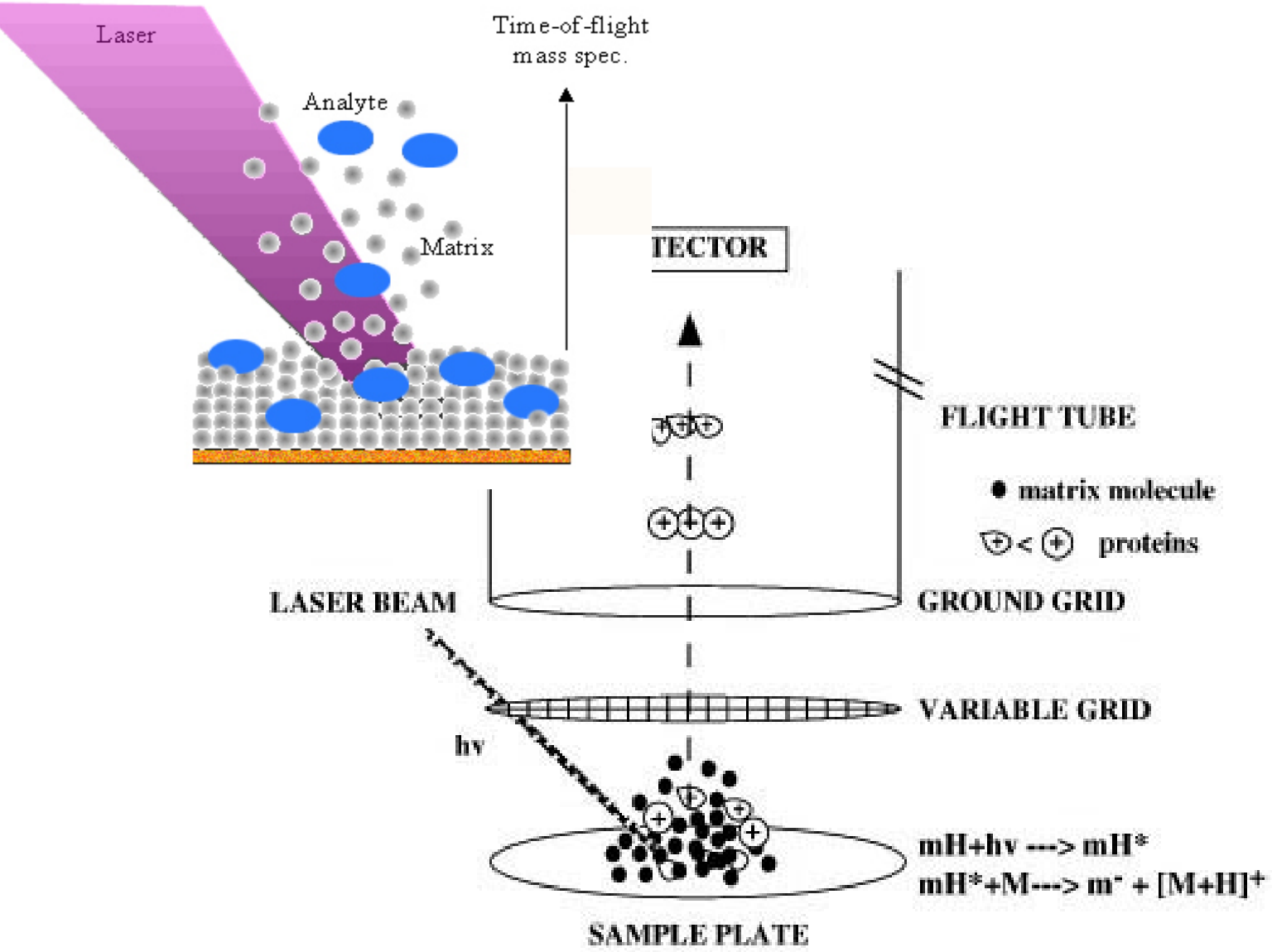
- 1980'lerin sonunda Alman ve Japon bir grup
- Proteinlerin **peptid kütle parmak izi**

Prensip

- MALDI kütle spektrometrede kullanılan bir **iyonizasyon tekniği**
- Biyomoleküllerin (protein, peptid ve şeker gibi) büyük organik moleküllerin (polimer, dendrimer, makromolekül gibi) analizi
- İyonizasyon **lazer atışı**
- **Matriks** biyomolekülün amacı; direkt lazer atışının tahribinden korumak ve iyonizasyonu kolaylaştırmak

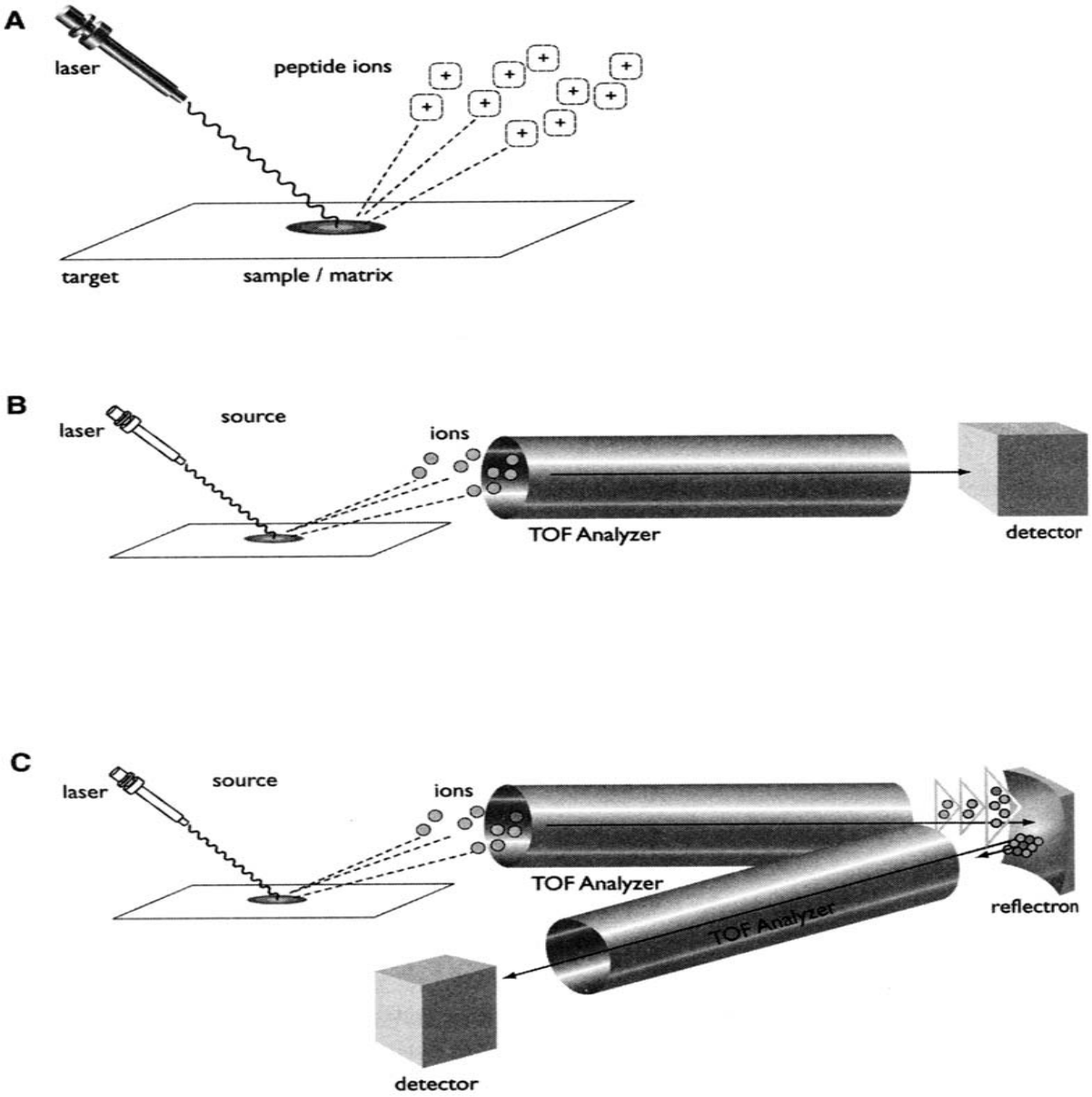
Matriks

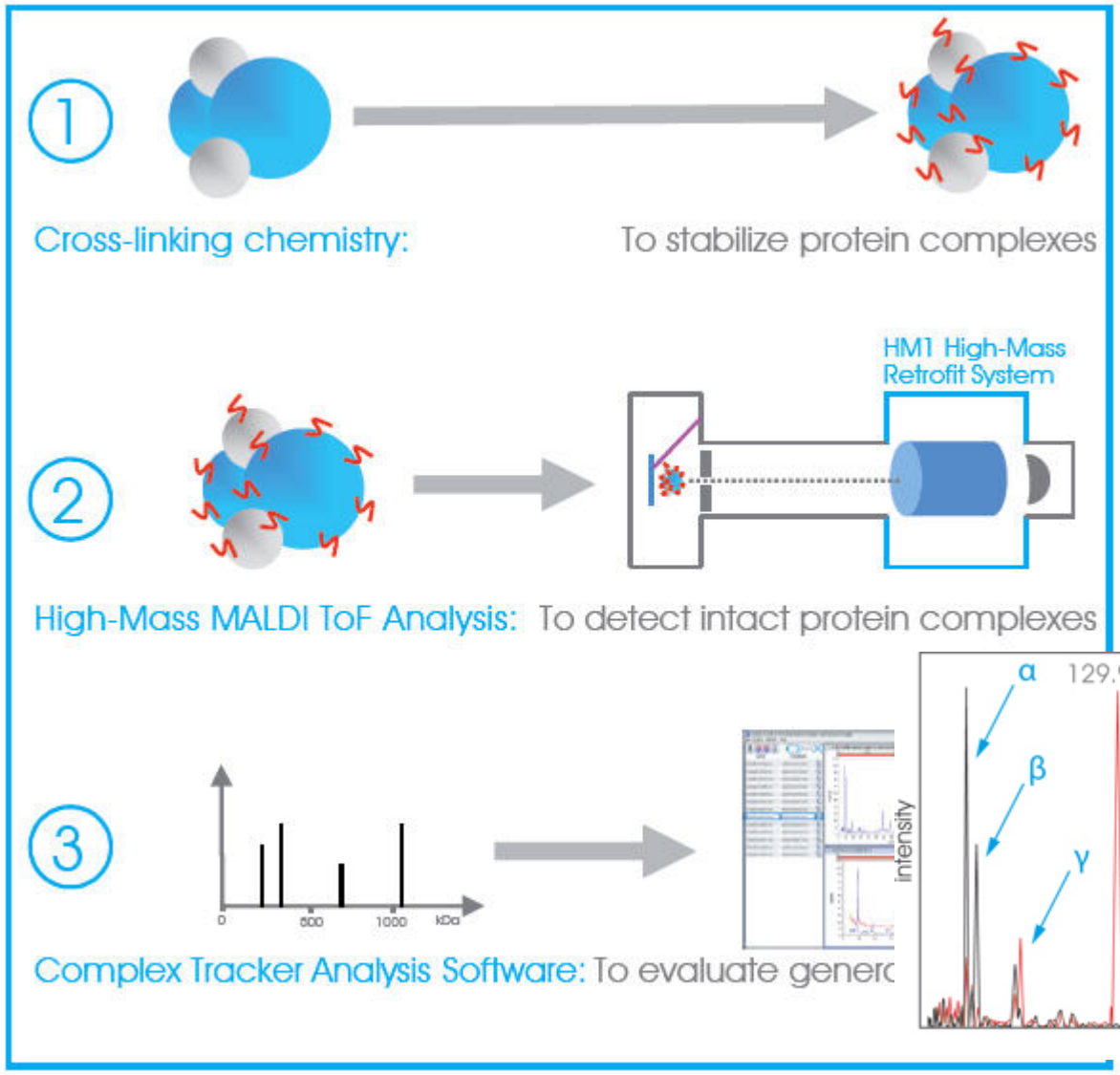
- UV absorbe eden matriks (Hillenkamp ve Karas)
- Matriks ve polimer moleküler düzeyde uygun bir çözücü (formik asit, trifloroasetik asit, asetik asit v.b.) içinde karıştırılır.
- Çözücü polimerin agregasyonunu engeller.
- Örnek/matriks karışımı örnek prop ucuna yerleştirilir.
- Vakum koşullarıyla çözücü uzaklaştırılarak matriks molekülleri içinde homojen olarak yayılmış polimer moleküllerini bırakır.


Laser
Time-of-flight
mass spec.
Analyte
Matrix
TECTOR
FLIGHT TUBE
matrix molecule
proteins
LASER BEAM
GROUND GRID
VARIABLE GRID
hv
mH+hv ---> mH*
mH*+M---> m⁻ + [M+H]⁺
SAMPLE PLATE

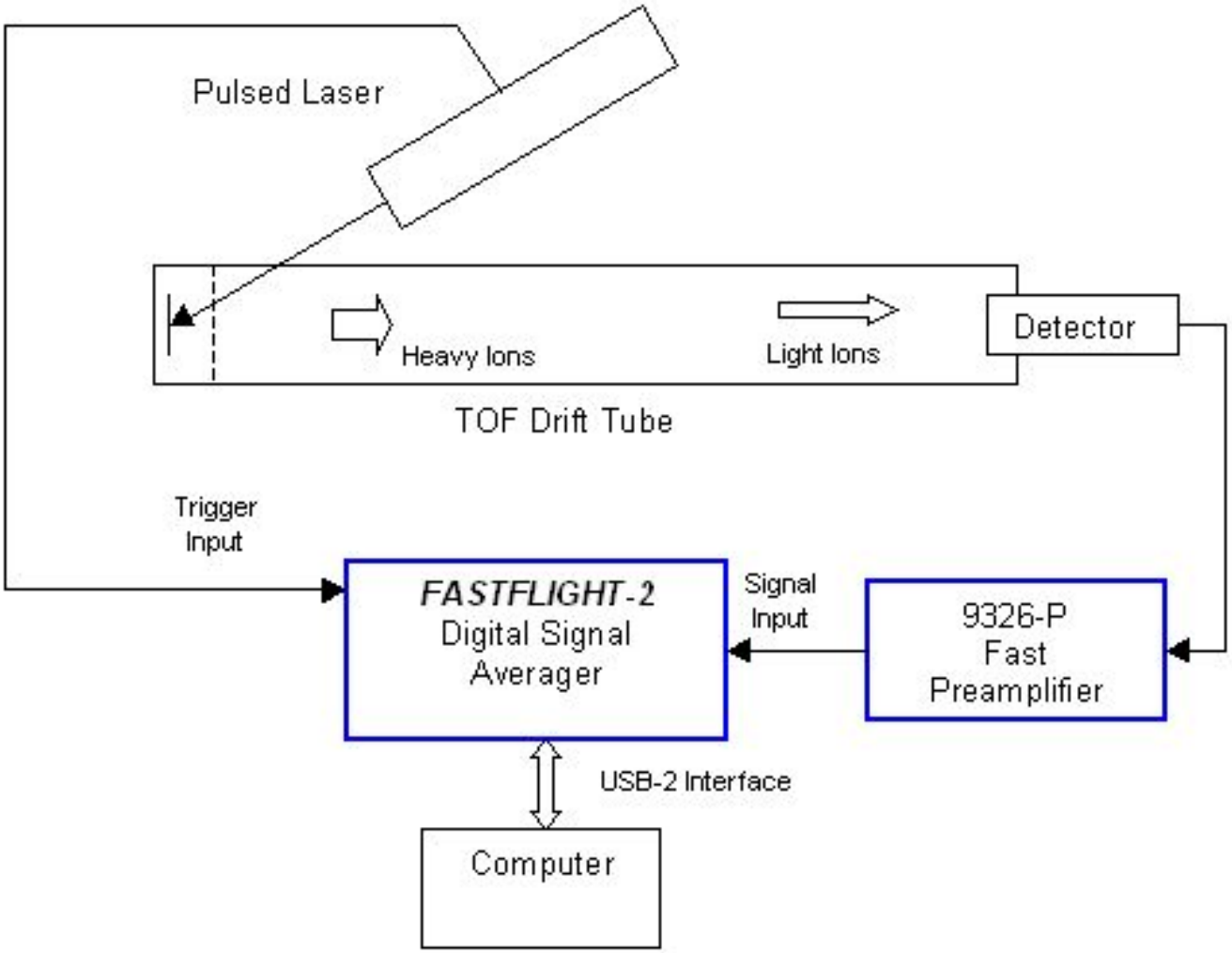
Lazer vuruş atımları uygun frekansa ayarlanınca, kısmen buharlaşmış ve buhar fazında intakt polimeri taşıyan ve polimer zincirlerinin elektrik yüklenmesine neden olan matrikse enerji aktarılır.

MALDI-TOF Kütle spektrometre



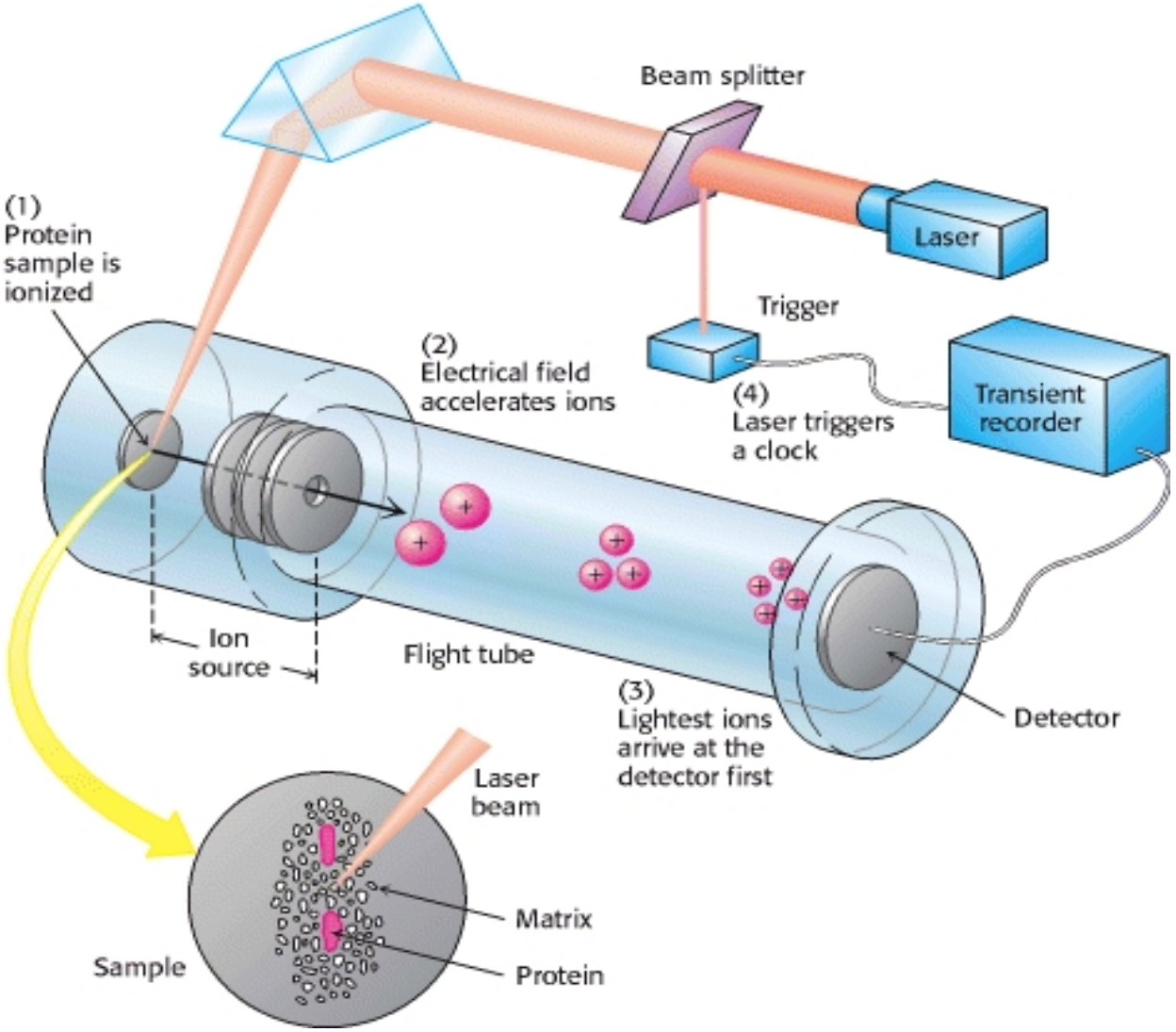


Protein Complex Analysis by High-Mass MALDI ToF MS


Pulsed Laser
Heavy Ions
Light Ions
Detector
TOF Drift Tube
Trigger
Input
FASTFLIGHT-2
Digital Signal
Averager
Signal
Input
9326-P
Fast
Preamplifier
USB-2 Interface
Computer

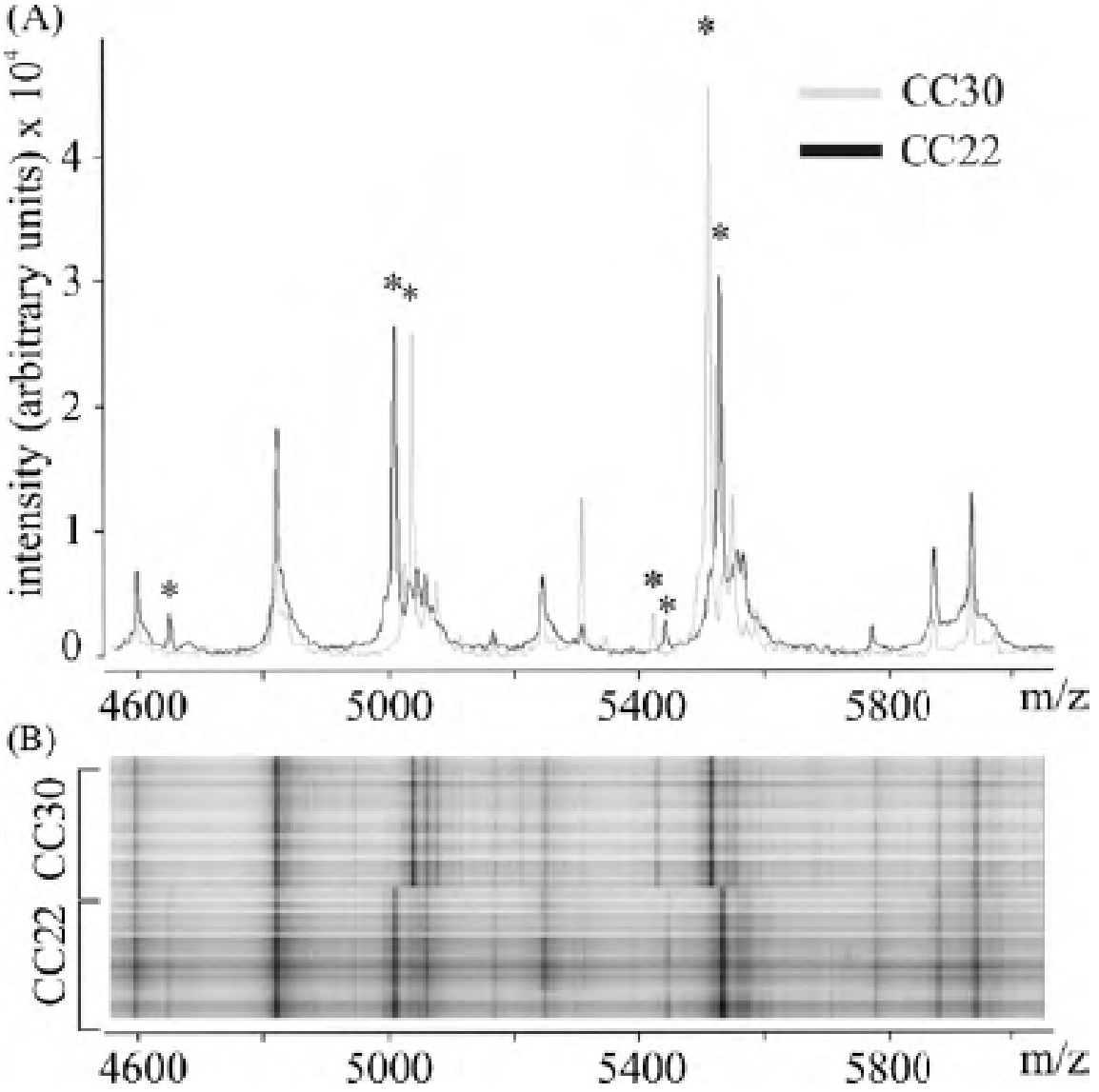
Biraz fizik...

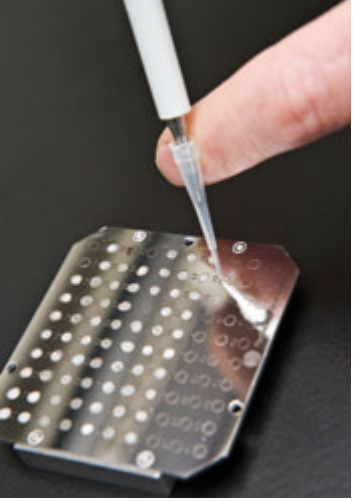
- Lazer vuruşları ile oluşan dijitalize veriler **TOF kütle spektrumu** oluşturacak şekilde toplanır.
- TOF kütle spektrumu zamanın bir fonksiyonu olarak saptayıcı sinyalin bir kaydıdır.
- Kütlenin (m) bir molekülünün uçuş zamanı ve bu mesafeyi geçerken yüklendiği akım (z) $(m/z)^{1/2}$ye orantılıdır.
- Zaman ve $(m/z)^{1/2}$ arasındaki ilişki **iyonların kütlelerini** hesaplamada kullanılabilir.


Beam splitter
Laser
(1)
Protein
sample is
ionized
Trigger
(2)
Electrical field
accelerates ions
(4)
Laser triggers
a clock
Transient
recorder
Ion
source
Flight tube
(3)
Lightest ions
arrive at the
detector first
Detector
Laser
beam
Matrix
Protein
Sample

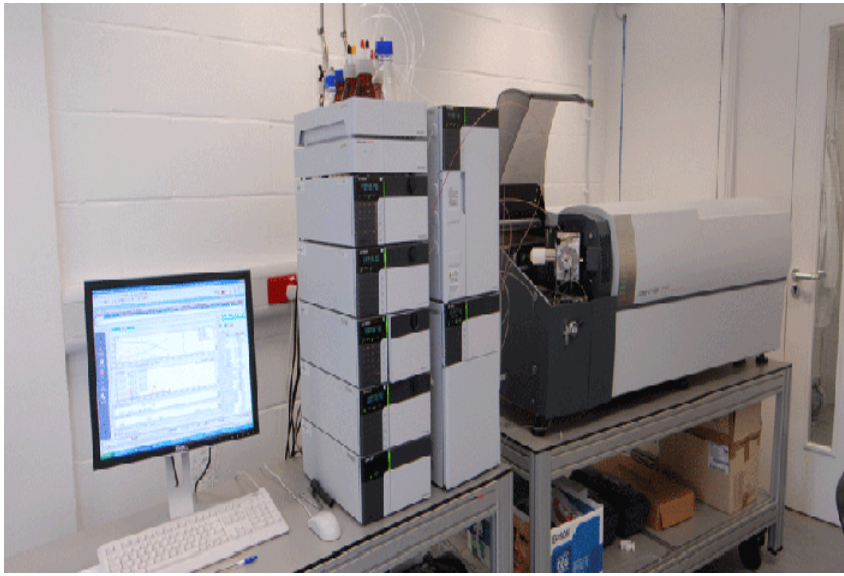
Biyoinformatik Analiz

- BioTyper (Bruker Daltonics Inc., Bremen, Almanya)
- Saramis (AnagnosTec GmbH, Potsdam-Golm, Almanya)


(A)
intensity (arbitrary units) x 10^4
0
1
2
3
4
CC30
CC22
*
*
*
*
*
4600
5000
5400
5800
m/z
(B)
CC30
CC22
4600
5000
5400
5800
m/z



Applied Biosystems Voyager DE Pro MALDI-TOF
Applied Biosystems Q-Star ESI-Quadrupole-TOF
Shimadzu LC MS IT-TOF mass spectrometer
Sequenom Mass ARRAY Compact Analyser



Belirleyiciler

- Diagnostik
- Prognostik
- Prediktif

“MALDI-TOF”	8396
“MALDI-TOF” “bacteria”	1393
“MALDI-TOF” “fungi”	847
“MALDI-TOF” “virus”	249

http://www.ncbi.nlm.nih.gov/sites/entrez

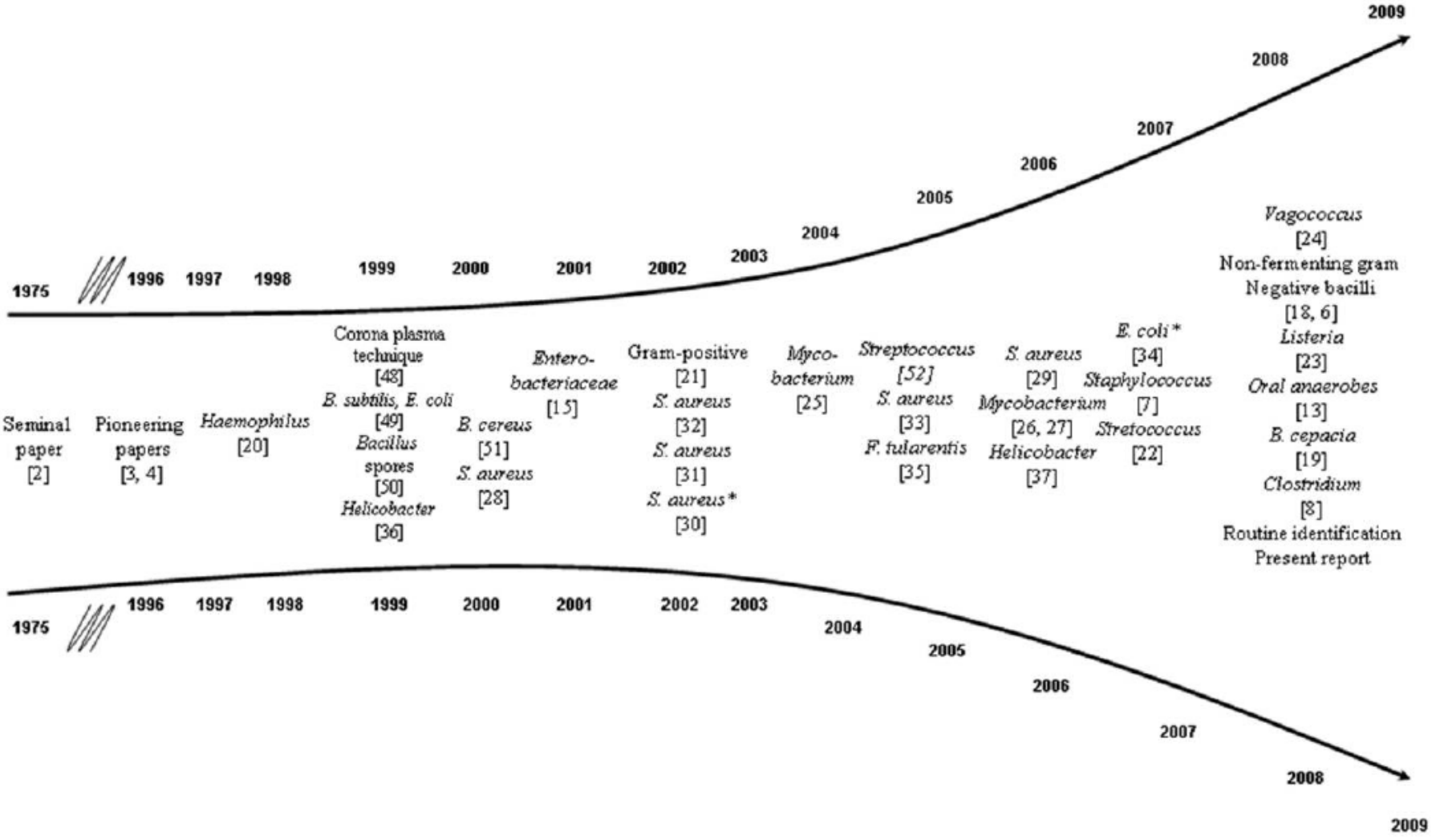
Kasım 2010

- MALDI-MS mikroorganizma analizi için önemli bir tanı aracı
- MALDI-MS
  - hastalık monitorizasyonu
  - tanısı
  - kanın taranması
- Bakteri, maya ve filamentöz mantarların, virus, viral vektörler ve doğru ve hızlı tanımlanması
- Taksonomi ve epidemiyoloji

MALDI-MS
BAKTERİ

- m/z oranı ile biyobelirleyici iyonların belirlenmesi
- Isıtılan **intakt** bakteri hücreleri

Anhalt JP, Fenselau C. Identification of bacteria using mass spectrometry. Anal Chem **1975; 47:219–25.**


1975
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
Seminal paper [2]
Pioneering papers [3, 4]
Haemophilus [20]
Corona plasma technique [48]
B. subtilis, E. coli [49]
Bacillus spores [50]
Helicobacter [36]
B. cereus [51]
S. aureus [28]
Entero-bacteriaceae [15]
Gram-positive [21]
S. aureus [32]
S. aureus [31]
S. aureus* [30]
Myco-bacterium [25]
Streptococcus [52]
S. aureus [33]
F. tularentis [35]
S. aureus [29]
Mycobacterium [26, 27]
Helicobacter [37]
E. coli* [34]
Staphylococcus [7]
Stretococcus [22]
Vagococcus [24]
Non-fermenting gram Negative bacilli [18, 6]
Listeria [23]
Oral anaerobes [13]
B. cepacia [19]
Clostridium [8]
Routine identification
Present report

Rapid Commun Mass Spectrom. 1996;10(10):1227-32.

Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry.

Holland RD, Wilkes JG, Rafii F, Sutherland JB, Persons CC, Voorhees KJ, Lay JO Jr.

Food and Drugs Administration, National Center for Toxicological Research, Jefferson, AR 72079, USA

RAPID COMMUNICATIONS IN MASS SPECTROMETRY, VOL. 10, 883–888 (1996)

Detection of Pathogenic and Non-pathogenic Bacteria by Matrix-assisted Laser Desorption/ Ionization Time-of-flight Mass Spectrometry

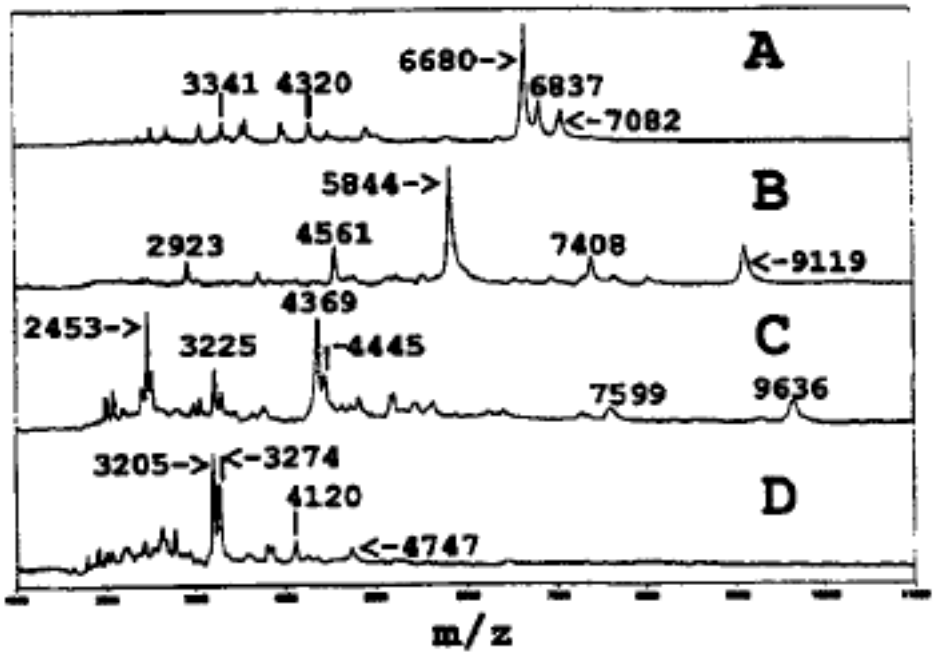
T. Krishnamurthy,* P. L. Ross and U. Rajamani
U.S. Army Edgewood RD&E Center, APG, MD 2101(

Bacillus anthracis

Brucella melitensis

Yersinia pestis

Francisella tularensis

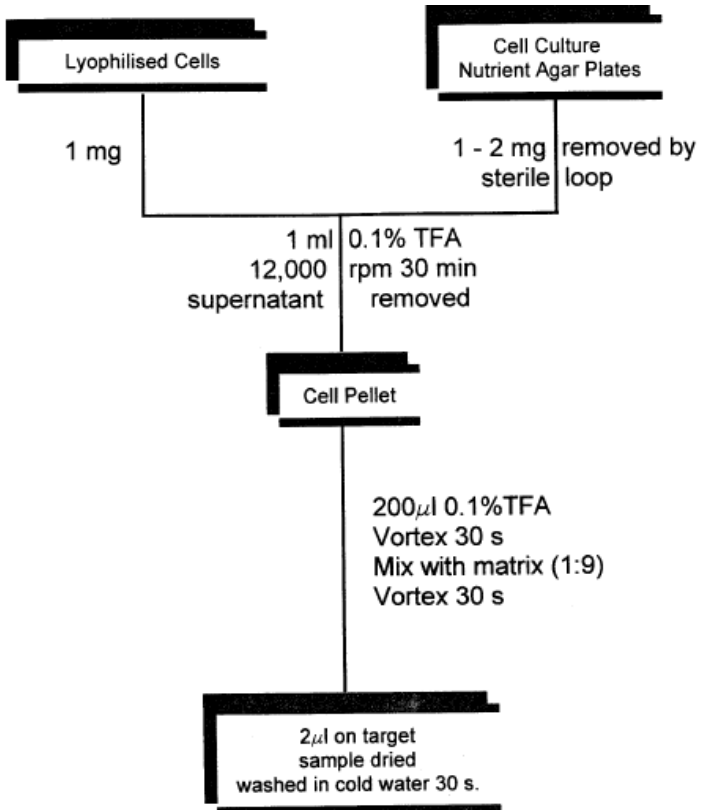


RAPID COMMUNICATIONS IN MASS SPECTROMETRY, VOL. 12, 176–180 (1998)

The Characterization of Micro-organisms by Matrix-assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry

Kevin J. Welham,[1]* Mark A. Domin,[1] D. Eoin Scannell,[1] E. Cohen[2] and David S. Ashton[1]

[1]ULIRS Mass Spectrometry Laboratory, Department of Pharmaceutical and Biological Chemistry, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WCIN 1AX, UK
[2]Faculty of Pharmacy, University of Lyon, Lyon, France.

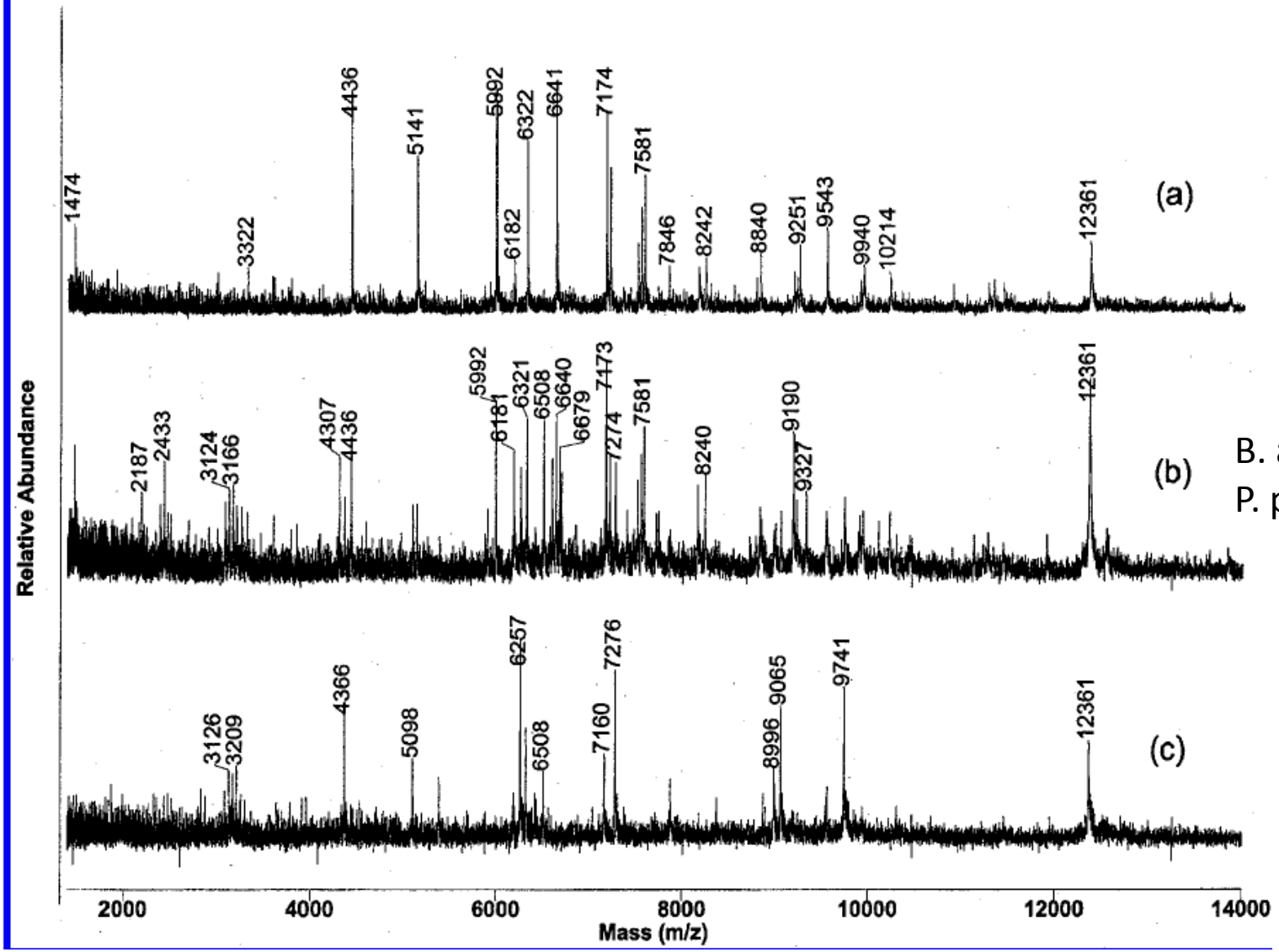


- Gram-pozitif koklar
  - Stafilokok, streptokoklar
- Enterobacteriaceae
  - *Escherichia coli*
  - *Yersinia enterocolitica*
  - *Erwinia* türleri
  - *Salmonella enterica*
- Nonfermenter bakteriler
- Campylobacter
- *Helicobacter pylori*
- Aeromonas
- *Haemophilus influenzae*
- *Streptococcus pneumoniae*
- *Streptococcus agalactiae*
- *Bartonella henselae*
- Neisseria, Listeria, mikobakteri
- Antibiyotik direncinin dakikalar içinde belirlenmesi

Anal. Chem. **2000,** *72,* 1217−1223

An Algorithm for Automated Bacterial Identification Using Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

Kristin H. Jarman,* Sharon T. Cebula, Adam J. Saenz, Catherine E. Petersen, Nancy B. Valentine, Mark T. Kingsley, and Karen L. Wahl



P. putida

B. atrophaeus, E. coli, P. putida

E. coli

>%90 doğru tanımlama

Ongoing Revolution in Bacteriology: Routine Identification of Bacteria by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

Piseth Seng,[a] Michel Drancourt,[a] Frédérique Gouriet, Bernard La Scola, Pierre-Edouard Fournier, Jean Marc Rolain, and Didier Raoult

Clinical Infectious Diseases 2009; 49:543–51

1660 bakteri izolatının **%95.4 doğru** tanımlanmış

1 izolat için 6 dakika

Maliyet konvansiyonel fenotipik tanımlamanın **%22-32**

COLONY

MALDI-TOF

GRAM-STAINING

Identification and stop

Delay 6 min

Cost 2.44 €

Manual susceptibility test

Semi automated susceptibility test

Delay: 5 min

Cost: 0.60 €

Current methods of identification

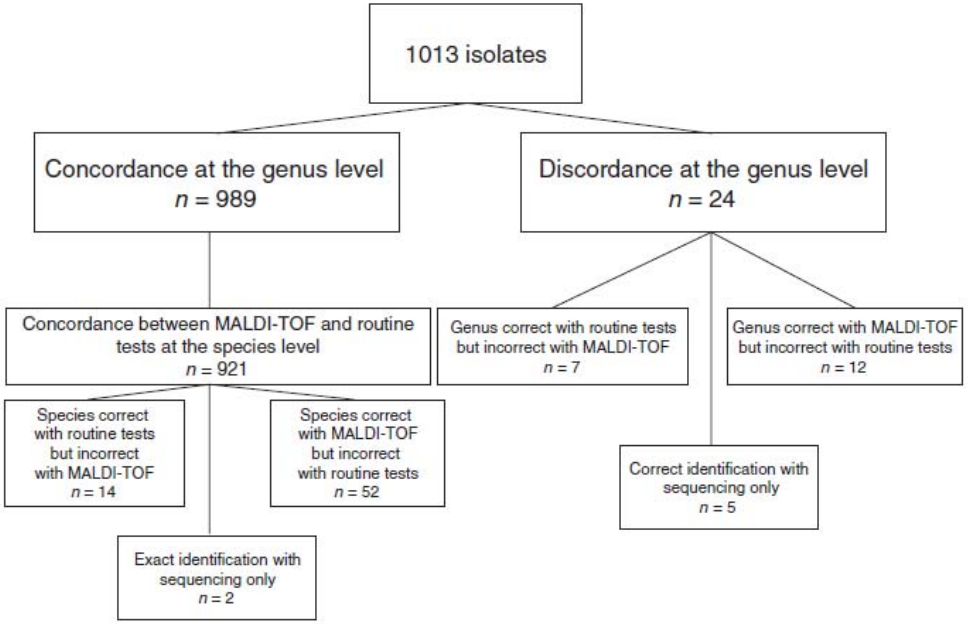
Current methods of identification and susceptibility tests

Total delay	6 min	24-28 h	24-48 h	5-48 h	5-48 h
Total cost	2.44 €	6.60-7.40 €	9.04-9.84 €	4.60-13.85 €	11.2-15.45 €

Matrix-assisted laser-desorption/ionization BIOTYPER: experience in the routine of a University hospital

E. Bessède[1,2,3], M. Angla-gre[1], Y. Delagarde[1], S. Sep Hieng[1], A. Ménard[1,2,3] and F. Mégraud[1,2,3]

Mayıs 2010



MALDI-TOF %99
Fenotipik yöntem %98

MALDI-TOF Mass Spectrometry identifies 90% of bacteria directly from Blood Culture vials

Authors: Wardi Moussaoui1, Benoit Jaulhac1, Anne-Marie Hoffmann1, Markus Kostrzewa2,Philippe Riegel1, Gilles Prévost1*

Ağustos 2010

MALDI-Biotyper

213 Gram (-) 193 % 91.08

319 Gram (+) 284 %89.02

(*Streptococcus pneumonia- S.mitis*)

MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory

Etienne Carbonnelle [a,c,*], Cécile Mesquita [a], Emmanuelle Bille [b,c], Nesrine Day [a], Brunhilde Dauphin [d], Jean-Luc Beretti [b], Agnès Ferroni [b], Laurent Gutmann [a,c], Xavier Nassif [b,c]

Clinical Biochemistry 2010

Summary of major studies using MALDI-TOF for bacterial identification. GN: Gram negative, GP: Gram positive.

Authors	Sample		Id species level	Id genus level	Main identification difficulty	Comments
Seng et al. [32]	Routine ($n=1660$)	all routine samples	83.8%	95%	*Propionobacterium acnes*, *Streptococcus pneumoniae*, *Stenotrophomonas maltophilia*, *Shigella* sp.	First line method of identification
van Veen et al. [36]	Routine ($n=980$)	all routine samples	92%	98.8%	*Streptococcus pneumoniae*, anaerobic bacteria	
Blondiaux et al. [37]	Routine ($n=362$)	all routine samples	72.9%	87%	viridans streptococci group. *Shigella* sp.	
Prod'hom et al. [42]	Blood ($n=126$)	positive blood culture	77.8%, GN: 89.1%, GP: 71.6%	78.7%, GN: 89.1%, GP: 72.9%	*Streptococcus mitis* group, *Staphylococcus* sp.	The presence of a capsule explain partially the low identification rate of *S. pneumoniae*, *H. influenzae*, *K. pneumoniae*
La Scola et al. [43]	Blood ($n=599$)	positive blood culture	76%	76%	*Streptococcus* sp., polymicrobial samples	
Stevenson et al. [44]	Blood ($n=212$)	positive blood culture (179), spiked bottles (33)	80.2%	80.2%	*Streptococcus mitis* group, *Propionobacterium acnes*	
Ferroni et al. [45]	Blood ($n=685$)	positive blood culture (388), spiked bottles (312)	89%	98%	*Streptococcus pneumoniae*, *Streptococcus mitis* group	For mixed culture, most abundant germ was in most cases identified. Fast method
Christner et al. [46]	Blood ($n=277$)	positive blood culture	94.2%	95%	Cocci Gram +	Mismatching mostly resulted from insufficient bacterial count and occurred preferentially with Gram +
Ferreira et al. [47]	Blood ($n=300$)	positive blood culture	42.6%, GN: 83.3%, GP: 31.8%	71.6%, GN: 96.6%, GP: 65.7%	*Streptococcus mutans*, *Staphylococcus* sp., *Staphylococcus aureus*	No mixed culture
Ferreira et al. [48]	Urine ($n=220$)	positive urine samples	91.8%, GN: 93.6%, GP: 66.6%	92.7%, GN: 94.6%, GP: 66.6%	*Streptococcus* sp., *Enterococcus* sp., *Raoultella* sp.	Best results with high bacterial account $>10^5$ CFU/mL, *E. coli* $>10^5$ CFU/mL: 97.6% correct id rate, 5 mixed cultures: 3 identifications

MALDI-MS
MANTAR

Filamentöz mantarlar

- Penicillium
- Aspergillus
- Fusarium
- *Trychophyton rubrum*, *T. interdigitale*, *T. tonsurans*
- *Arthroderma benhamiae*

MALDI-TOF Mass Spectrometry for fast and accurate identification of clinically relevant *Aspergillus* species

Alexandre Alanio[1], Jean-Luc Beretti[1], Brunhilde Dauphin[1], Emilia Mellado[2], Gilles Quesne[1], Claire Lacroix[3], Anouar Amara[1], Patrick Berche[1], Xavier Nassif[1], Marie-Elisabeth Bougnoux[1*]

Haziran, 2010

140 izolat (138) **%98.6**

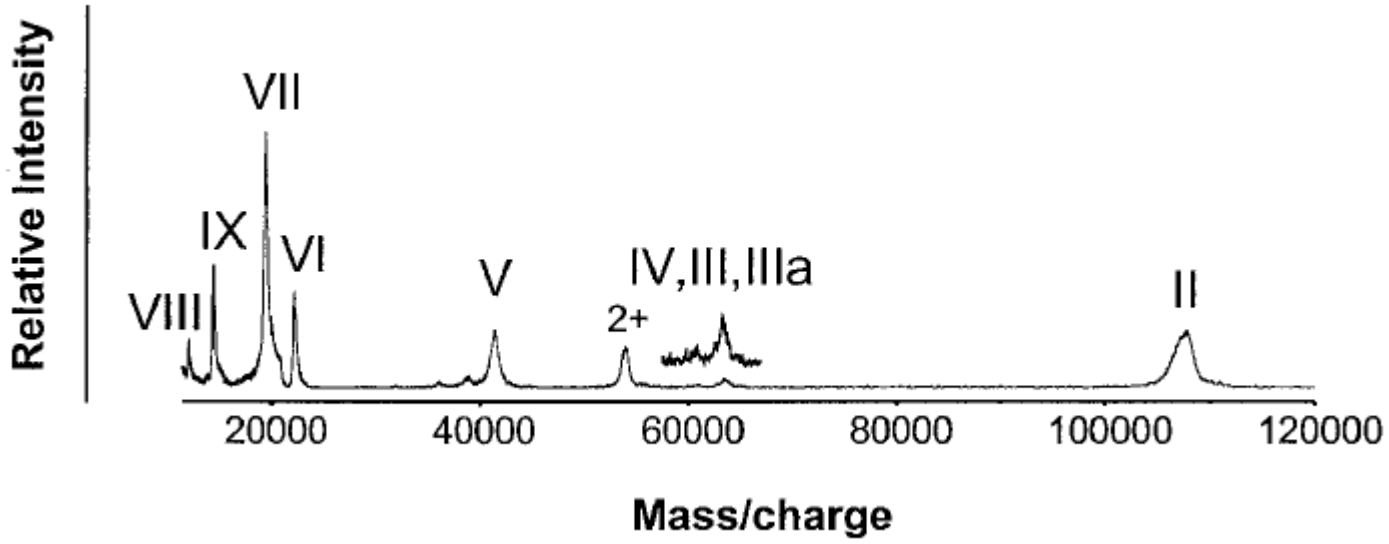
MICROORGANISMS DIRECT IDENTIFICATION FROM BLOOD CULTURE BY MALDI-TOF MASS SPECTROMETRY.

Laura Ferreira1*Fernando Sánchez-Juanes1*Isabel Porras Guerra2
Mª Inmaculada García García2 José Elías García Sánchez2
José Manuel González-Buitrago1,4#Juan Luis Muñoz Bellido2,5#
Nisan 2010

Gram (-) %96.6
Gram (+) %65.7
İnvaziv fungal enfeksiyonlarda üzerinde çalışılmalı

MALDI-MS
VİRUS

- Viral yapısal proteinlerin belirlenmesi
- Gen terapi için değiştirilen viral vektörlerin izlenmesi
- Terapötik gen ve ekspresyon ürününün belirlenmesi



Adenovirus yapı proteinleri

Yao X, Freas A, Ramirez J, Demirev PA, Fenselau C. 2001.Proteolytic O-18 labeling for comparative proteomics: model studies with two serotypes of adenovirus. Anal Chem 73:2836±2842.

SONUÇ

- Bakteri identifikasyonu yüz güldürücü
  - Dakikalar içinde
  - Maliyet etkin
  - Antimikrobiyal direnç
  - İlaç hedefleri
- Mantar çalışmaları
  - Filamentöz mantarlar