PROTEOMSKA ANALIZA S HIV OKUŽENIMI MAKROFAGI

ULA ŠTOK
MIRJAM KMETIČ
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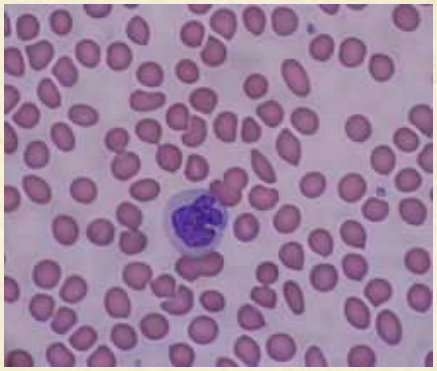
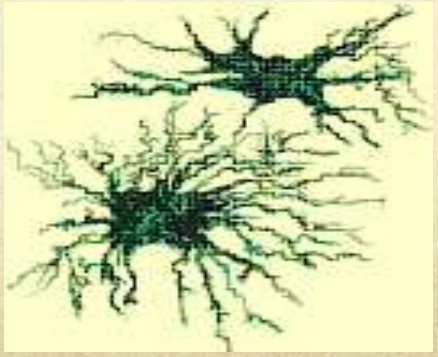
HIV

MAKROFAGI



MONOCITI



MIKROGLIJE

SELDI (surface-enhanced laser desorption/ionization)

PROTEOMSKE

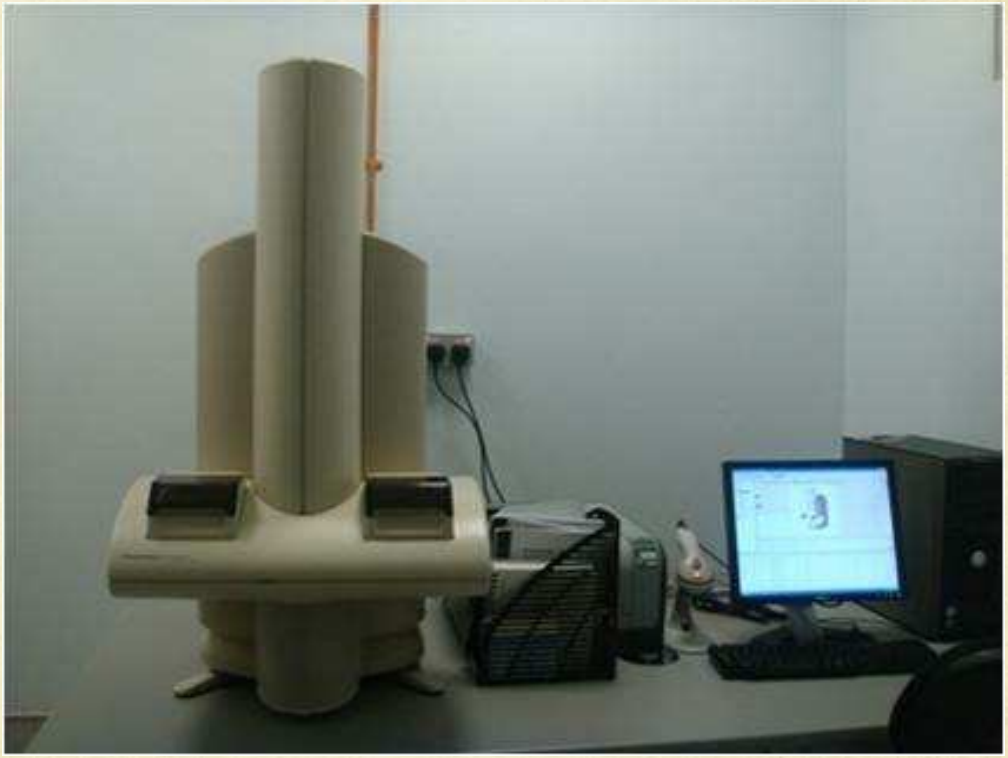
TEHNIKE:

*LC-MS/MS

*1D elektroforeza

*SDS page

*MALDI



SISTEM DVEH TEHNIK

KROMATOGRAFIJA

MASNA SPEKTROMETRIJA

BIOMARKERJI:

- **cistatin B**

- beta 2 mikroglobulin

- neopterin

- HLA (human leukocyte antigen)

- **VZORCI:**

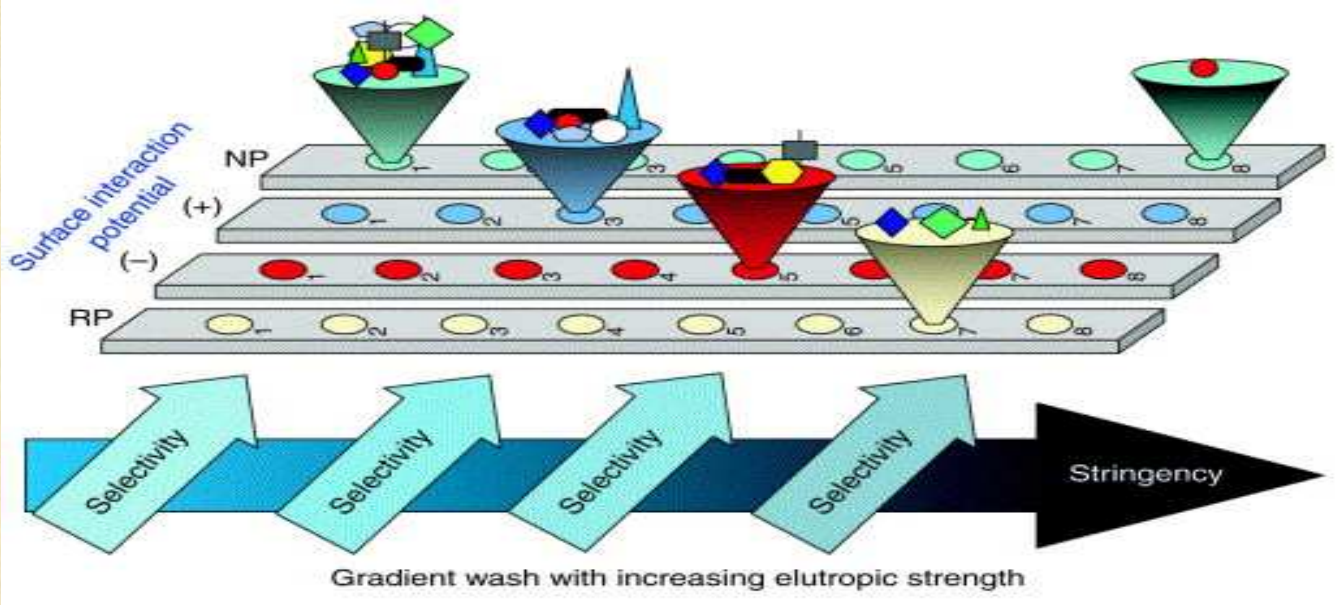
- KRI

- URIN

- CEREBRALNA TEKOČINA

ZGRADBA APARATA

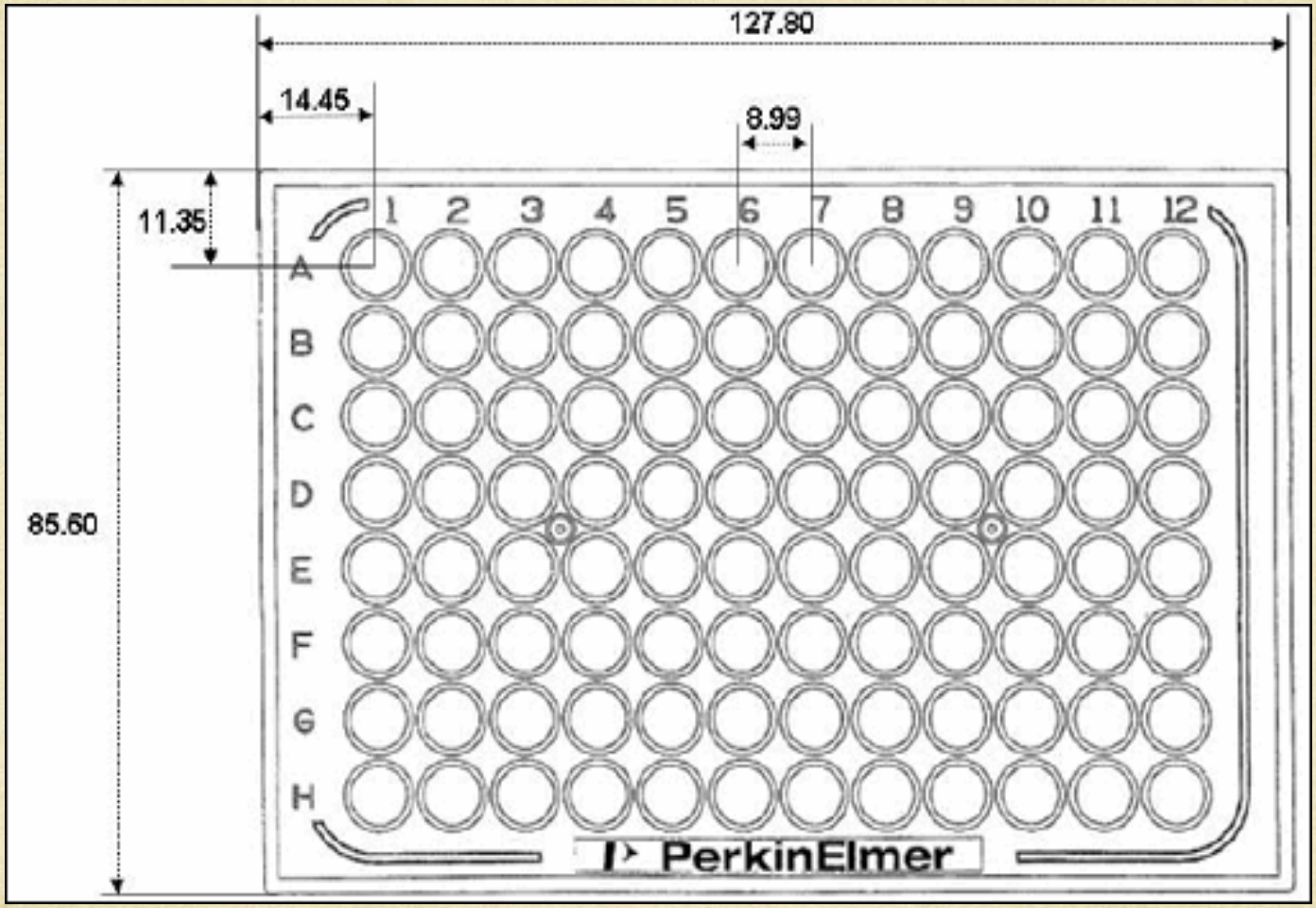
IZVAJAMO LAHKO RAZLIČNE TIPE KROMATOGRAFIJE:



*ANIONSKA
*KATIONSKA
*AFINITETNA
*HIDROFOBNA
*HIDROFILNA

BIOLOŠKE MOLEKULE:

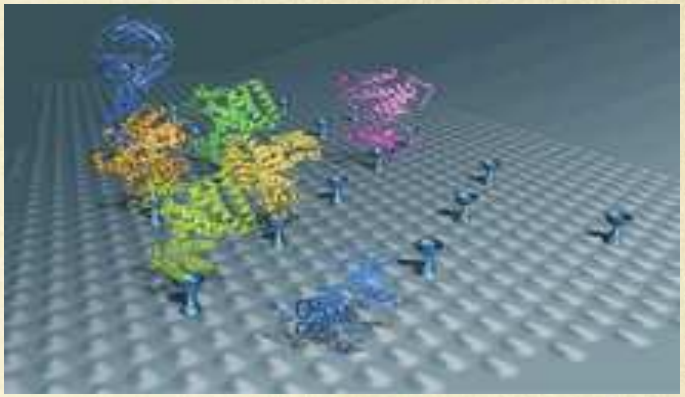
*PROTITELESA
*RECEPTORJI


127.80
14.45
8.99
11.35
85.60
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
PerkinElmer

1. KORAK

•IZBIRA NOSILCA
•NANAŠANJE VZORCA

2. KORAK

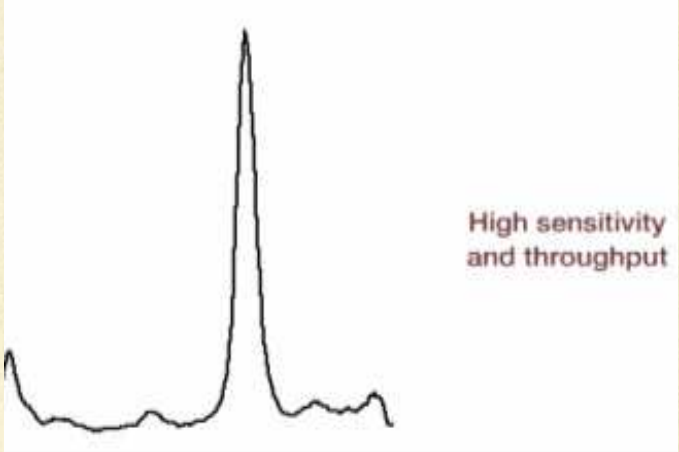


*INKUBACIJA
*SPIRANJE S PUFROM
*NANOS EAMS (energy absorbing molecules)

3. KORAK

*OBSEVANJE Z LASERJEM

4. KORAK



*PROGRAMSKA ANALIZA PODATKOV

Poskus

- Placentalnimakrofagiuspešnejeomejujejor eplikacijovirusa HIV
- Intracelularnimehanizmizaviranjanisopozn ani
- Študijatemeljinaprimerjaviproteomovokuž enih in neokuženihmakrofagov
- Uporabarazličnihmetod

Potekposkusa

- Pridobitevplacentalnih in monocitnihmakrofagov
- Okužbadelaceličnihkultur s HIV-1
- 4 vzorci: okuženi in neokuženiplacentalni in monocitnimakrofagi
- Analizaprofilaproteinovceličnihlizatovs SELDI-TOF

Uporaba SELDI-TOF

- Šibkakationskaizmenjava CM-10 in močnaanionskaizmenjava Q-10
- KalibracijaProteinChipbralnikazavsakoanalizo
- CM-10 čipi so dalivišjevrednosti in intenzitetevrhov
- Spektri, ki se razlikujejo v 99 vrhovih
- GEE (Generalized estimating equation) statističneanalize in številneprilagoditveprimerjav
- 27 proteinov

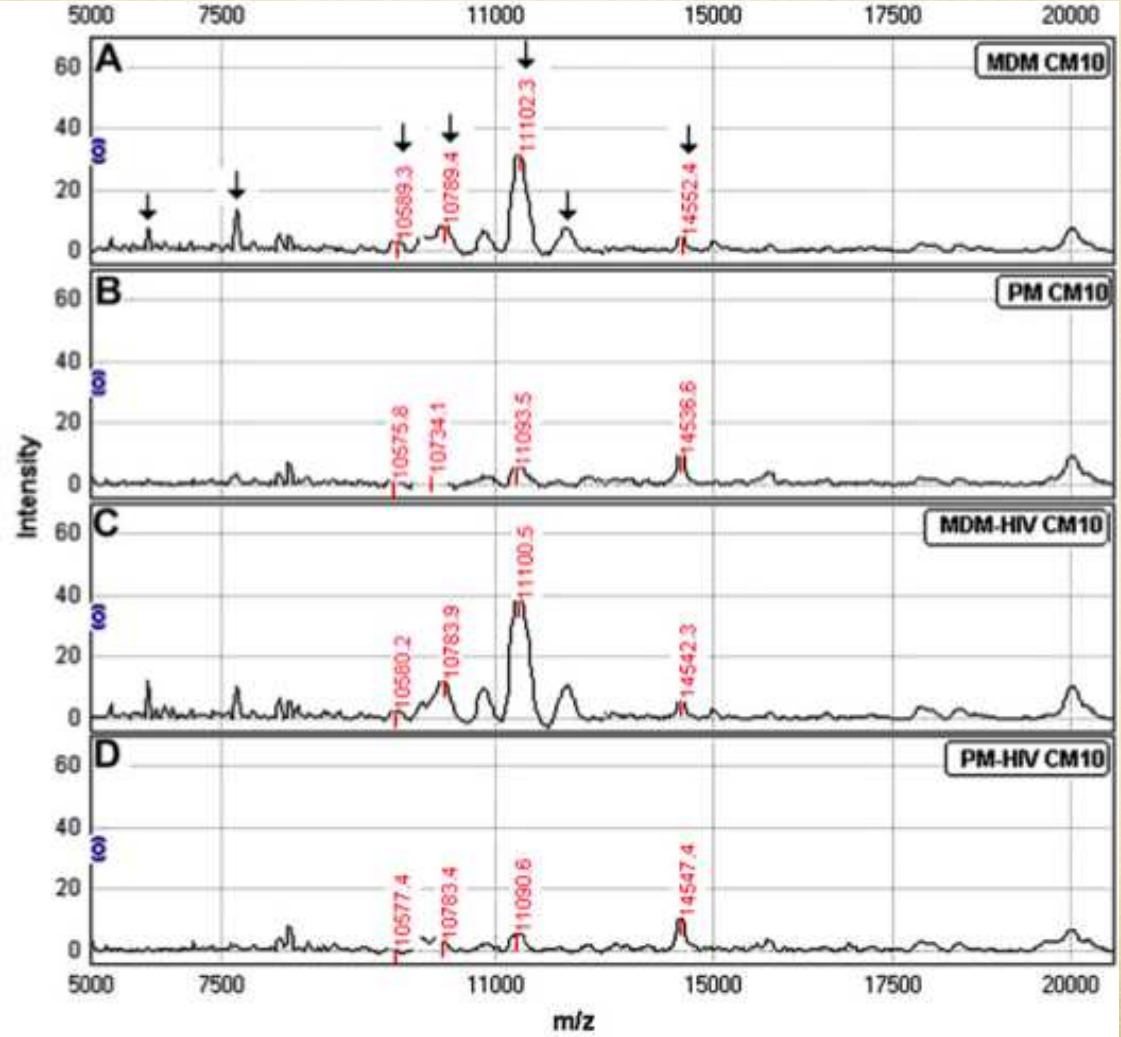
27 različnoizraženihproteinov

SELDI-TOF protein peaks in uninfected and HIV-infected PM

Peak number	Peak median, m/z[a]	Peak intensity in PM[b]	Peak intensity in PM-HIV[c]
1	5037	↑	↑
2	5232	↑	↑
3	5644	–	↓
4	5680	↓	↓
5	5815	–	↓
6	5913	↓	↓
7	7699	↓	↓
8	7890	↑	–
9	9193	↓	↓
10	9550	–	↓
11	9905	↓	↓
12	10,098	↓	↓
13	10,216	↓	↓
14	10,330	–	↓
15	10,582	–	↓
16	10,776	↓	↓
17	10,959	↓	↓
18	11,101	↓	↓
19	11,289	↓	↓
20	11,878	–	↑
21	12,214	↑	↑
22	12,344	↑	↑
23	12,430	↑	↑
24	13,623	↑	↑
25	14,545	↑	–
26	15,013	↓	↓
27	15,790	↑	↑

Luciano-Montalvo C et al., Proteomic Analyses Associate Cystatin B with Restricted HIV-1 Replication in Placental Macrophages, Placenta (2008)

SELDI-TOF spekter



Luciano-Montalvo C et al., Proteomic Analyses Associate Cystatin B with Restricted HIV-1 Replication in Placental Macrophages, Pla

Identifikacijaproteinov

- LC-MS/MS peptidnosekvenciranje je boljobčutljivo, specifično, zagotovinatančnepodatke o identifikacijiproteina in številčnosti
- Uporaba 1D SDS PAGE in izrezustreznihlis
- Sekvenciranje z LC-MS/MS
- Primerjavateoretičnihmolekulskih mas proteinov z vrhovi SELDI-TOF
- Identifikacija 12 proteinov

12 identificiranihproteinov

Comparison of SELDI protein peaks and LC–MS/MS sequencing data

SELDI-TOF, m/z[a]	Molecular weight[b]	Total peptides detected in sequencing[c]				Protein name[d]
		MDM	PM	MDM-HIV	PM-HIV	
9905	9987	NF	2	NF	NF	Cytoskeletal 14-like protein
10,330	10,438	2	NF	NF	NF	SH3 glutamic acid rich-like protein 3
10,776	10,835	NF	NF	2	NF	Protein S-100 A8 (calgranulin A)
10,959	10,932	2	2	2	2	10 kDa heat shock protein
11,101	11,139	4	2	4	3	Cystatin B
11,289	11,749	2	2	2	2	Cytochrome C
12,344	12,774	4	NF	4	NF	SH3 glutamic acid rich-like protein 1
12,430	12,895	NF	2	2	NF	Myotrophin
13,623	13,242	NF	NF	2	2	Protein S-100 A9 (calgranulin B)
14,545	14,716	NF	NF	2	NF	Galectin-1
15,013	15,054	5	6	5	5	Profilin
15,790	15,936	2	NF	NF	2	Superoxide dismutase CuZn

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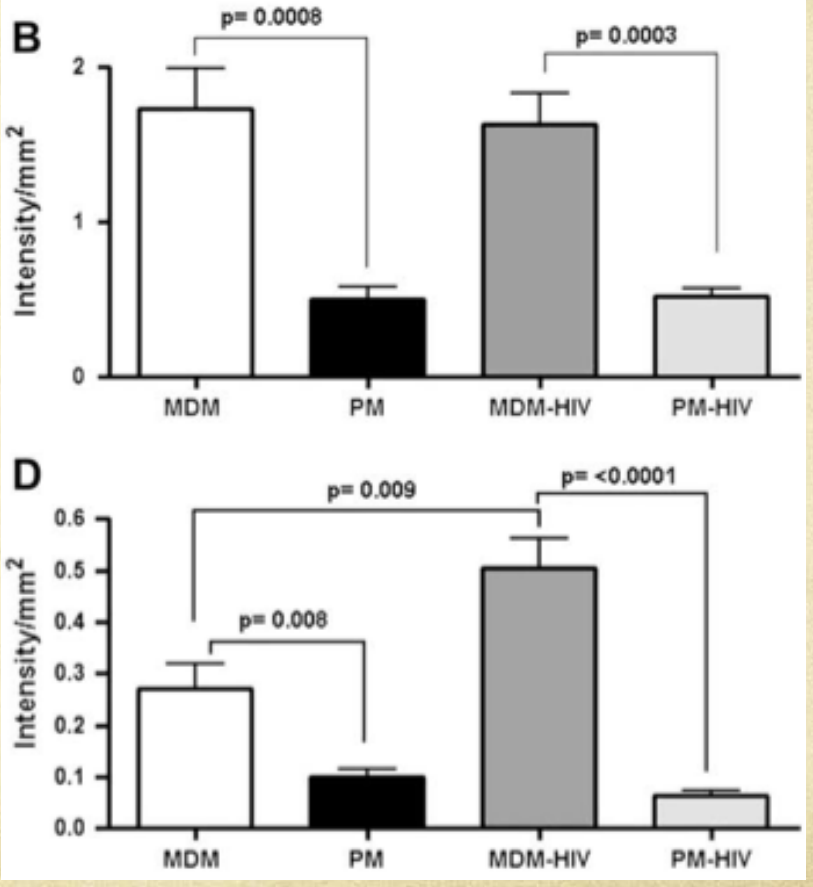
- Izmedtehizberejocistatin B zanadaljnjeanalize

Prenoswestrn

- Prenoswestrnpokaževi šjinivocistatina B primonocitnihmakrofagi h



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Rezultati

- Različnoizražanjecistatina B v placentalnih in monocitnihmakrofagih
- Nižjinivocistatina B bi lahkopripomogel k omejevanjureplikacije HIV
- Sintetičniinhibitorjicistatina B bi lahkopomagalinadzorovatiokužbo s HIV

HVALA ZA VAŠO POZORNOST J

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