High resolution MALDI plates for the direct coupling of PAGE separations and tissue analysis with MALDI mass spectrometry

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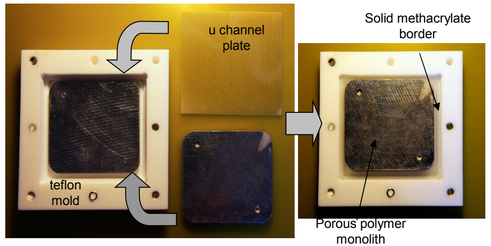
Introduction

High-resolution, 3-dimensional MALDI-TOF plates[1] developed as a direct interface between PAGE separations and tissue samples with MALDI mass spectrometry. Construction uses µ-channel plates composed of 25 µm ID collimated-holes structures (CHS) filled with monolithic chromatography media. Plates operate by capturing and concentrating sample (protein / peptides) in the porous-structured, hydrophobic plate interior. After capture, material is eluted back to the surface using organic solvents containing MALDI matrix. Upon drying, analytes are incorporated into matrix crystals on the plate surface. Finally, the plate is loaded directly into the mass spectrometer for analysis.

Methods

Plate Construction

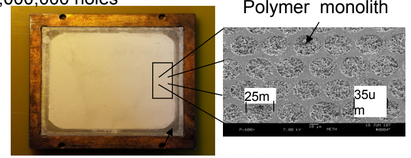
-Micro channel plates piranha solution cleaned and silanized (2% methylacryloxypropyl-trimethoxysilane, 95% ethanol)
-Two phase polymer plate construction done in teflon mold with UV polymer initiation. Solid outer methacrylate border, Porous inner butylmethacrylate monolith[2] active surface (40% polymer, 60 % porogen, 0.4% initiator)



-Stainless steel plates block UV light during solid border construction
-SS plates removed for interior monolith construction
-excess polymer is removed with a razor blade

Collimated-Hole Structure (CHS) Capture Plate

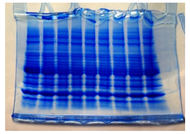
~10,000,000 holes

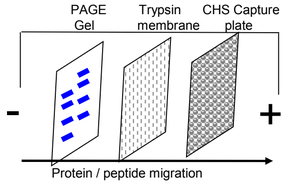


Solid methacrylate border

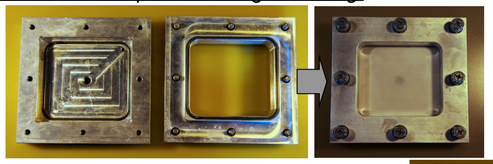
PAGE-Gel Interface

-CHS plate takes place of peptide capture membrane in “Molecular Scanner”[3,4]
-PAGE separated protein is blotted through trypsin membrane and captured on CHS plate





Peptide elution and plate washing / cleaning



-Aluminum housing with o-ring seal allows for washing (salt removal) , plate cleaning and regeneration enabling for multiple use

-Sample elution for MS analysis accomplished by forcing matrix in organic solution through one side of the plate and then drying eluent on the opposite side

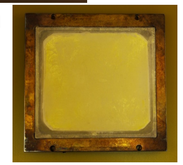
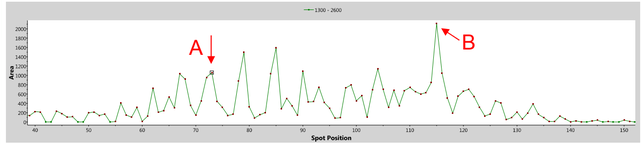


Plate with matrix

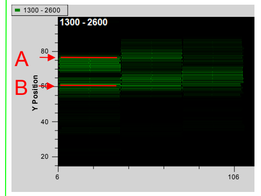
Mass Spectrometry

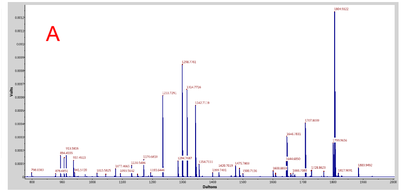
- Ion Current plotted below Vs. Spot Position.
- 18195 spectra of 200 laser shots.
- kHz laser, scanning 1.2 mm / sec.
- m/z range 150 – 3000.
- 0.2 mm per spectrum in y (direction of electrophoresis).
- 1 mm per spectrum in x.
- All Spectra mapped to a spot, 1 mm x 35 mm.
- -> 252 averaged spectra (84 rows, 3 columns).

Results

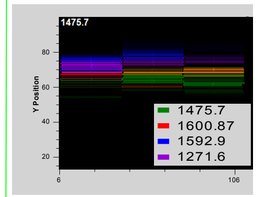


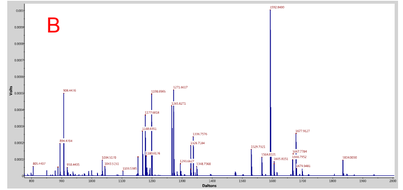
84 rows, 3 columns





Distribution of total ion current





Colored heat map:
Distribution of 4 masses across the plate

PMF Search Conditions

- Peptides m/z 800-4000
- Proteins 50-3000 aa in length
  - 16293 cow proteins in all
- Matching (ChemPlex, see ref. 5)
  - Tolerance
    - 4 ppm minimum
      - (no additional credit ppm below this)
    - <8 ppm (top peak(s))
    - <20 ppm (all peaks)
  - 1 peak in most intense 100 with Ch > 2
  - 2 peaks matched in all
  - More credit with intensity
  - More credit for matching expected peptides
    - %ChS matched as filter
    - Arg peptides >> Lys peptides
    - Weighted scale for missed cleavages
- 117 peaks detected in A

Symbol key

•MB	myoglobin
•HBB	hemoglobin beta
•ALDOA*	fructose bisP aldolase
•TPM2*	tropomyosin
•ACTA2*	actin

IRank	Intensity Rank	Sc	Score
Int	Intensity	%ChM	% ChemScore Matched
Ch	ChemScore	% IntM	% Intensity Matched
# pep	# peptides;	PrRank	Protein Rank
length	#aa in protein		

Spectrum A data

protein data						
symb	#pep	length	Sc	%ChM	%IntM	PrRank
MB	5	154	490	49.1	44.7	1
HBB	4	145	98	42.4	13.1	2

peptide data						
symb	IRank	Sequence	mz	Int	Ch	ppm
MB	1	VEADVAGHGQEVLLR	1592.8	636	20.0	0.2
MB	2	LFTGHPETLEK	1271.7	215	1.8	-1.5
MB	2a	HLAESHANKHK	1271.7	215	0.4	0.7
MB	4	GHHEAEVK	906.4	151	2.0	4.6
HBB	5	LLGNVLVVVLAR	1265.8	132	20.0	-3.2
HBB	6	VVAGVANALAHR	1177.7	114	20.0	1.1
HBB	11	VKVDEVGGEALGR	1328.7	46	7.0	0.7
HBB	70	VVAGVANALAHRYH	1477.8	2	2.0	12.6
MB	74	HGNTVLTALGGLLK	1393.8	1	2.0	-7.4

Spectrum B data

protein data						
symb	#pep	length	Sc	%ChM	%IntM	PrRank
ALDOA	9	364	54	33.3	18.9	1
TPM2	9	284	13	26.5	15.4	2
ACTA2	4	377	7	25.6	1.0	3

peptide data						
symb	IRank	Sequence	mz	Int	Ch	ppm
TPM2	2.0	KLVLLEGELER	1298.8	376	6.0	5.8
ALDOA	3.0	ADDGRPFPQVLK	1342.7	251	20.0	-0.1
ALDOA	6.0	YSHEELAmATVTALR	1707.8	178	20.0	-19.7
ALDOA	8.0	RLQSLGTENTEENR	1646.8	111	6.7	-16.2
ALDOA	8a	LQSLGTENTEENRR	1646.8	111	3.9	-16.2
TPM2	12.0	KYEEVAR	894.5	34	10.5	-14.5
TPM2	12a	YEEVARK	894.5	34	2.0	-14.5
ALDOA	13.0	AAQEEYVK	937.5	33	2.0	-12.6
TPM2	14.0	LVLLEGELER	1170.7	30	18.0	6.4
TPM2	18.0	ATDAEADVASLNRR	1488.7	25	2.0	4.3
ACTA2	21.0	GYSFVTTAER	1130.5	22	16.2	0.3
TPM2	37.0	RLQLVEEELDR	1399.8	6	5.3	-10.3
ALDOA	39.0	AAQEEYVKR	1093.6	6	2.0	-0.9
ACTA2	41.0	QEYDEAGPSLVHR	1500.7	5	20.0	3.6
ALDOA	49.0	QLLLTADDR	1044.6	3	16.0	-10.6
TPM2	55.0	TLDDLEDEVYAQKmK	1813.9	3	0.4	13.2
ACTA2	56.0	AGFAGDDAPR	976.4	3	20.0	5.3
TPM2	62.0	LDKENALDR	1073.6	2	6.6	12.2
ALDOA	72.0	zQYVTEKVLAAVYK	1671.9	2	0.2	19.8
TPM2	72a	LVLLEGELERSEER	1671.9	2	6.8	11.5
ALDOA	86.0	PWALTFSYGR	1197.6	1	2.0	14.3
ACTA2	111.0	LWHHSFYNELR	1501.7	1	20.0	12.0

Conclusions

- Can couple SDS gels to monolithic capture plates.
- Can elute peptides to surface using matrix solution.
- Can identify multiple proteins per SDS gel band.

References

1) Hattan SJ, Vestal ML (2008) Anal Chem.; 80 : 9115-9123.
2) D. S. Peterson, T. Rohr, F. Svec, J. M. J. Fréchet (2002) Anal. Chem.; 74 : 4081-4088.
3) Binz PA et al. (1999) Anal. Chem.; 71 : 4981-4988.
4) Nadler TK, et al. (2004) Anal. Biochem.; 332 : 337-348.
5) Parker KC (2002) Scoring Methods in MALDI Peptide Mass Fingerprinting.. JASMS; 13 : 22-39.

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