A new "Molecular Scanner" design for interfacing gel electrophoresis with MALDI-TOF

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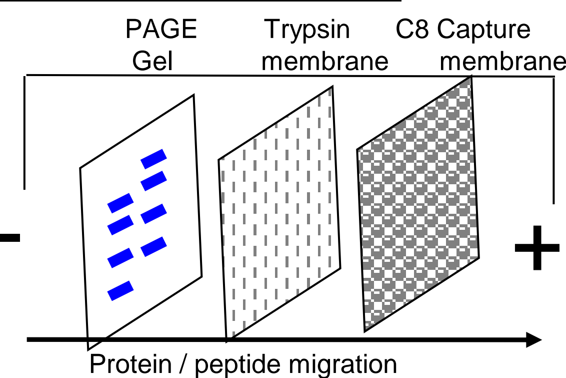
Stephen J. Hattan; **Kenneth C. Parker**; **Marvin L. Vestal** *SimulTof Corporation, Sudbury , MA*

Introduction

The concept of a “Molecular Scanner” introduced by Hochstrasser et. al.[1,2] was intended to provide an efficient mechanism for directly coupled protein gel electrophoresis with detection by MALDI mass spectrometry. Shown here is a new design for the “molecular scanner” incorporating novel membranes that demonstrate efficient capture and digestion of proteins blotted directly for gels. The resulting peptides are eluted from the membranes, detected by MALDI MS and interpreted by peptide mass fingerprinting[3] (PMF) with MS/MS confirmation. This work demonstrates the feasibility of this simple analytical workflow for doing top-down proteomics.

Molecular Scanner Concept



Old

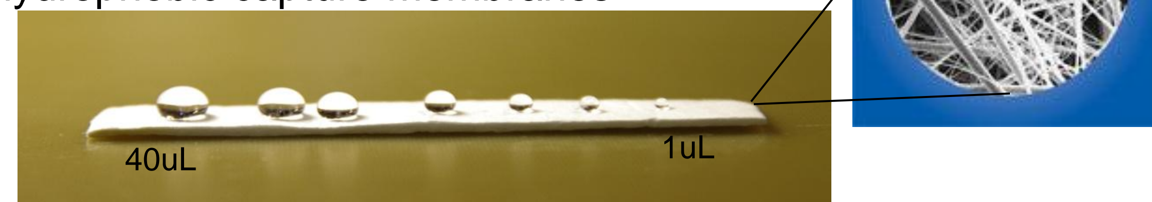
- -PAGE separated protein are orthogonally blotted from gel
- -Proteins pass through an immobilized enzyme membrane
- -Resulting peptides are captured on a hydrophobic membrane
- -Membrane is coated with Matrix and analyzed by MALDI MS

New

- -new membrane design using silianized glass filter paper
  - -efficient protein digestion
  - -high capacity peptide capture
- -captured peptide eluted from membrane prior to analysis

Membrane Construction

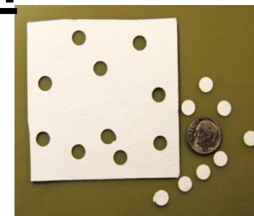
-Membranes are constructed by the silanization of commercial glass filter paper (Millipore, Pall).
-variety of silanes (Gelest Inc.) were used to create both immobilized enzyme and hydrophobic capture membranes

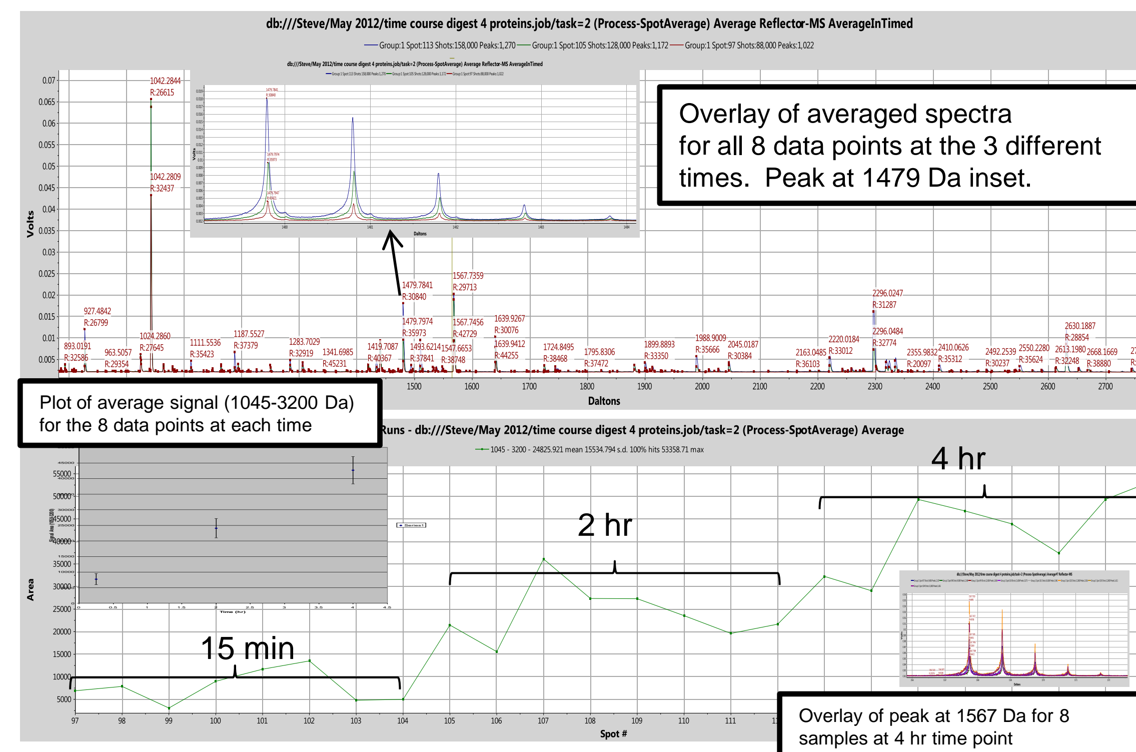


Membrane Characterization

Enzyme membrane digestion time

-membrane sampled randomly
-3 time points 15 min, 2 hr , 4 hr (BSA sample)
-4 samples each time point measured in duplicate

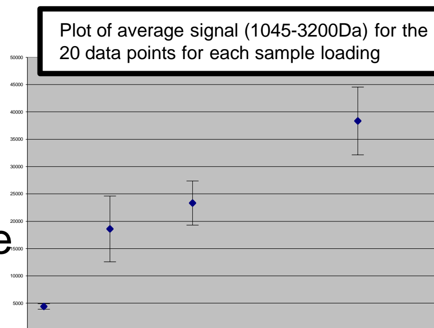


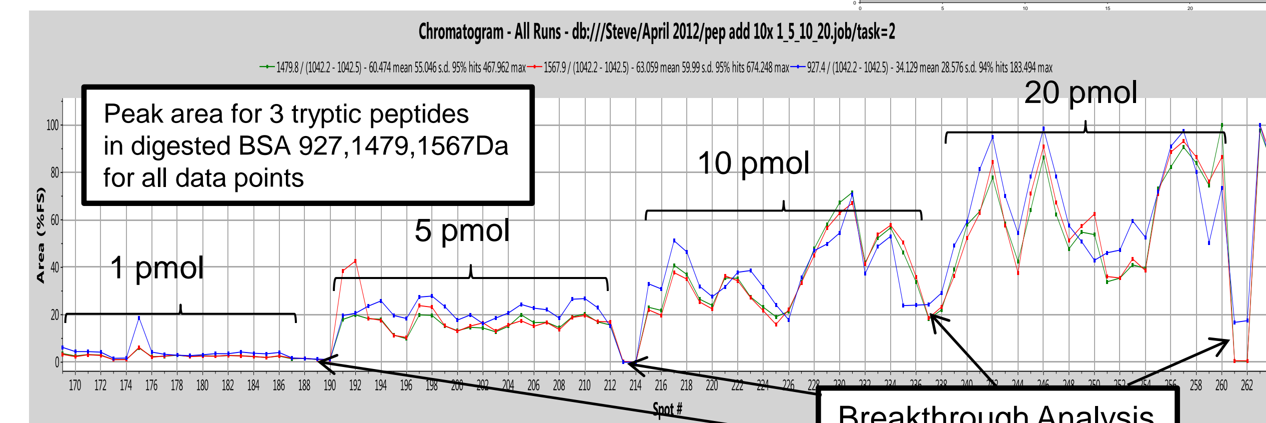


Protein digestion is efficient and reproducible but takes time

Capture membrane (C8) binding capacity

-membrane sampled randomly
-4 sample loads 1, 5, 10, 20 pmol digested BSA onto ~5mm² area
-10 sample each load measured in duplicate
-breakthrough collected and analyzed





5mm² area of membrane consistently binds 20pmol of digested protein with full peptide recovery

Blotting Experiment

-experiment run under normal Western blot conditions
-with and without our capture and digestion membrane

PVDF

No capture

Capture & digestion

Protein capture successful

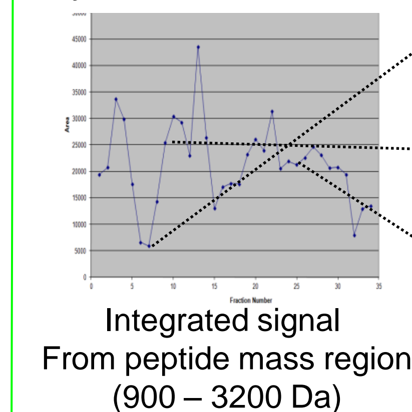
Protein transfer successful

No blotting load same for all lanes

4-12% gradient gel

1) yeast lysate loaded in all lanes
2) clear gel = blotting efficiency
3) with digestion/capture membranes, protein does not reach PVDF
4) capture / digestion membrane removed washed and sliced
5) individual fractions eluted separately (75%Acetonitrile)
6) all fractions analyzed MALDI-TOF MS (next frame)

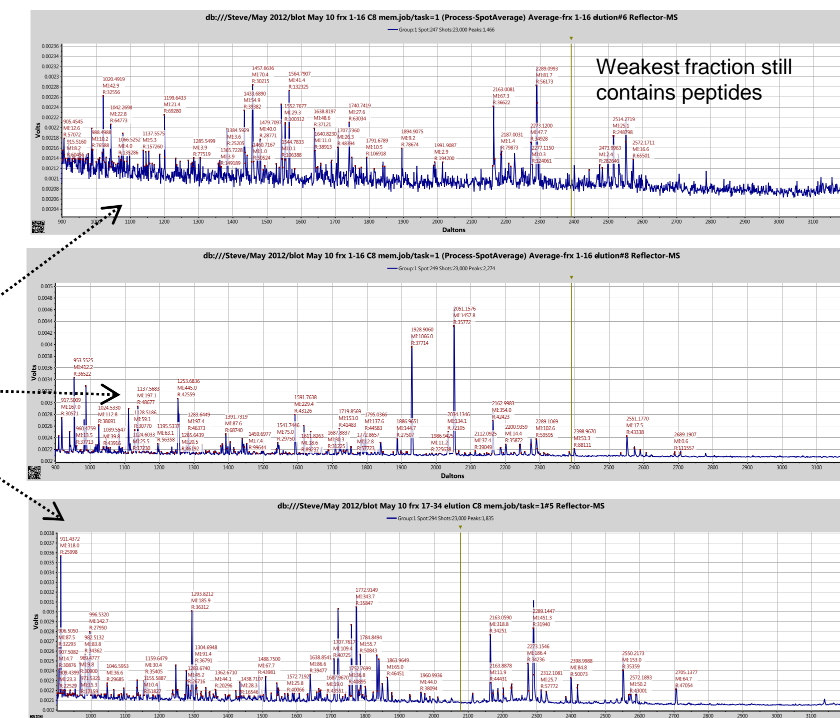
Capture Membrane processing



Lane sliced into 34 fractions

Results

34 fractions eluted and analyzed by MALDI-TOF MS



Integrated signal From peptide mass region (900 – 3200 Da)

Different fractions contain peptides originating from the proteins in that band --good for PMF--

Rank	Symbol	Organism	acc. #	AA length	Protein name	# peptides	TrSM	%CM	%IM	ppw
0	TRY_cow	cow	TRY1_BO	246	trypsin bovine	4	205137	71.7	5.5	2.4
1	RPL35A	Saccharo	P39741	120	60S ribosomal protein L35	5	107385	31.9	8.5	0.5
2	RPS24A	Saccharo	P26782	135	40S ribosomal protein S24	4	57720	21.2	5.8	0.5
3	RPL26A	Saccharo	P05743	127	60S ribosomal protein L26-A	3	36095	24.4	3.1	0.7
4	RPS17A	Saccharo	P02407	136	40S ribosomal protein S17-A	2	31083	24.0	2.8	1.8
5	RPL14B	Saccharo	P38754	138	60S ribosomal protein L14-B	2	21337	22.5	9.2	1.2
6	RPS16A	Saccharo	P40213	143	40S ribosomal protein S16	3	18959	38.9	3.4	7.7
7	RPL4B	Saccharo	P49626	362	60S ribosomal protein L4-B	4	11957	22.4	2.6	2.2
8	RPS19B	Saccharo	P07281	144	40S ribosomal protein S19-B	2	11695	18.7	8.6	0.8
9	RPS14A	Saccharo	P06367	137	40S ribosomal protein S14-A	3	11211	21.0	9.3	6.8

PMF results from fraction 9 (middle above) leads to multiple protein identifications ID’s confirmed by MS/MS

Conclusions & Future

Continue to improve membrane design
Automate membrane fractionation and elution

References

1) Binz P-A, et al. (2004) Current Opin in Biotech.; **15**: 17-23
2) Nadler TK, et al. (2004) Anal. Biochem.; **332**: 337-348.
3) Parker KC (2002) Scoring Methods in MALDI Peptide Mass Fingerprinting.; JASMS: **13**: 22-39.