A bi-functional glass membrane designed to interface SDS-PAGE separations of proteins with the detection of peptides by mass spectrometry

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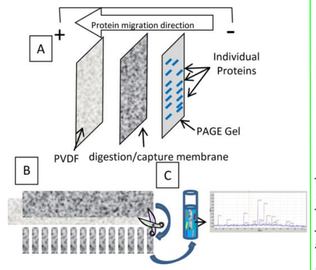
MP 006

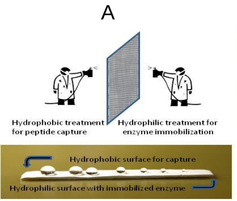
Introduction

We describe a digestion-capture membrane (DCM) that bridges the transition between electrophoretic separation of proteins (SDS-PAGE) and detection of peptides by MALDI. Proteins blotted into the membrane are digested into peptides, captured and concentrated while maintaining spatial resolution, and eluted into liquid optimized for mass spectrometry. Protein standards demonstrate 87% protein digestion precision, quantitative capture of ~ 20 pmol peptides in 25 mm^2 of membrane, and recovery of 83%. Following SDS-PAGE separation of yeast lysate and blotting onto the membrane, 72% of identified proteins were localized to a single fraction and 87% in 2 fractions (2 mm size, n=30). This workflow does not require specialized equipment, and is compatible with protein identification by protein mass fingerprinting (PMF) or MS/MS.

Methods

- Prepare bi-functional DCM from glass filter paper
- Run SDS gel.
- Blot onto DCM.
- Incubate DCM to promote digestion.
- Cut DCM into fractions.
- Elute.
- Gather MALDI spectra.
- Internally calibrate.
- Identify proteins by PMF using ChemApplex.





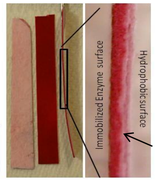


Fig. 1. **DCM Construction**: **(A)** Surfaces of 3 mm thick A/C glass filter paper are differentially treated with silane chemistry to produce a bifunctional DCM. One side is coated to enable enzyme immobilization; the other is treated to create a hydrophobic web.

(B) Ponceau S Dye Staining. The red surface indicates even staining by immobilized trypsin, whereas the pink surface indicates less staining of the hydrophobic surface. The cross section shows the evenness of the membrane layers.

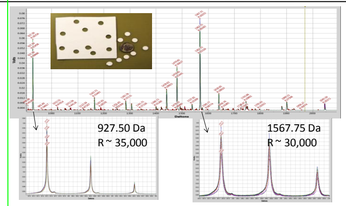


Fig. 2. **Reproducible Digestion**: Overlay of spectra from 10 parallel digests of BSA acquired from random sections of membrane. Inset: BSA tryptic peptides at m/z 927 and 1567. Photo: Membrane sections excised by hole-punching for all characterization studies.

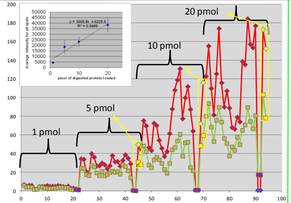


Fig. 3. **Peptide Binding Capacity:** 10 replicates of BSA digest loaded onto DCM at quantities of 1, 5, 10, and 20 pmol. Purple: Capture by PVDF membrane for each load (breakthrough apparent only in spots 91 and 92).Yellow: control samples; obtained from the 5, 10, and 20 pmol dilutions spotted directly onto MALDI plate (no membrane). Red: 1567 signal. Green: 927 signal. Inset: average intensity vs. the quantity loaded.

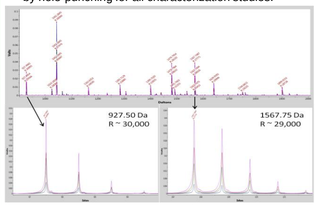


Fig. 4. **Linear Increase in Signal**: Overlay of spectral averages for all data points at four different sample loadings. Inset: BSA peaks at m/z 927 and 1567 Da.

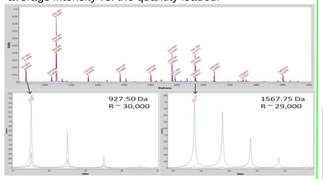


Fig. 5. **Peptide Binding Breakthrough:** Overlay of spectral average of 20 pmol load data with PVDF membrane placed behind the DCM to capture unretained peptides. Inset: m/z 927 and 1567 peaks. At 20 pmol loading quantity, m/z 927 begins to break through, while m/z 1567 is retained.

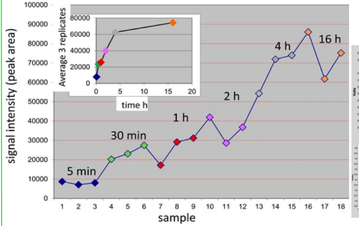


Fig. 6. **Time course of Enzyme Digestion**: Plot of the summed signal intensity for peptides in the mass range m/z 900−2500 resulting from membrane digestion of CytC. Digestion was quenched at different times in triplicate, as indicated by the color. Inset: average signal intensity for each set of replicates vs. time.

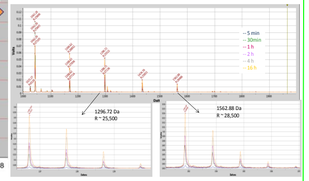


Fig. 7. **Digestion Time Course**: Spectral average of 3 replicates of CytC peptides eluted from DCM from m/z 900−2400 for the 5 min, 30 min, 1, 2, and 4 h and overnight digestion. Inset: peaks at m/z 1296 and 1562.

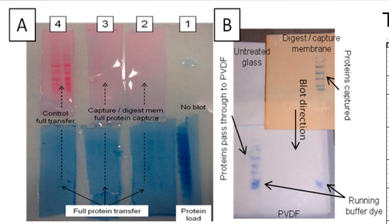


Fig. 8. **SDS-PAGE Gel and Membranes after Blot: (A)** Gel (Coomassie stain) and PVDF membrane staining (Ponceau S) after a blot of SDS-PAGE separation of yeast lysate. Section 1: a lane cut from the gel and stained (no blotting) , demonstrating protein separation. Sections 2 and 3: lanes blotted through the DCM A lack of protein bands in the gel and on the PVDF membrane demonstrates that proteins are quantitatively captured by the DCM. Section 4: lanes separated and blotted through untreated A/C glass filter paper. This section serves as a control, showing transfer of proteins out of the gel and their capture on PVDF. **(B)** DCM's ability to capture proteins and maintain resolution. Blotted standards are trapped by the DCM, but easily pass through an untreated glass membrane.

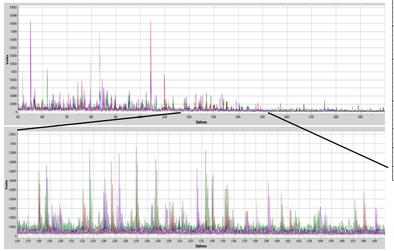
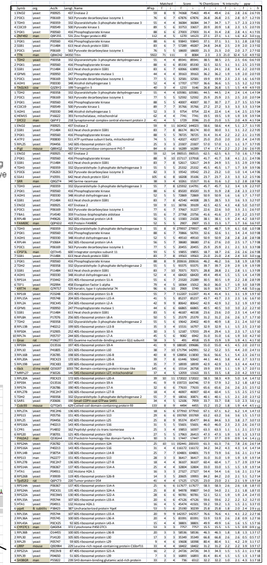


Fig. 9. **Fraction Complexity** : An overlay of 5 spectra obtained from 5 adjacent fractions of DCM following SDS-PAGE of yeast lysate. Inset: Region between mz 1160 an 1500. Note the DCM's ability to digest proteins into a rich mix of peptides while maintaining the spatial integrity of the separation.

Abbreviations: CytC, cytochrome C; BSA, bovine serum albumin; PVDF, polyvinylidene difluoride; DCM, digestion-capture membrane.

Table 1. Proteins vs. Fraction



Database derived from SwissProt: yeast (6582 proteins) combined with human, mouse, rat, cow, E. coli, B. subtilis, and Dictyostelium discoideum (69459 proteins in total). In this table, proteins have not been combined across fractions. Grey: protein clearly wrong. Most yeast proteins (other than grey ones) are well known to be abundant. Score, overall score from ChemApplex; %ChemScore, % of peptides matched based on ChemScore (heavily weighted toward arginine peptides; % intensity, % intensity matched' ppw, intensity weighted average ppm matched; f, final parameter value; i, initial parameter value prior to iterative subtraction.

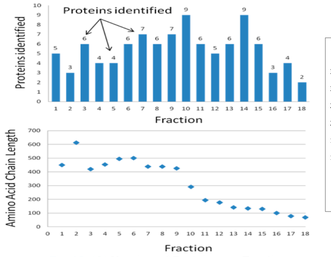


Fig. 10. **(A) Number of Proteins vs. Fraction**. **(B) Average Protein Length vs. Fraction.**

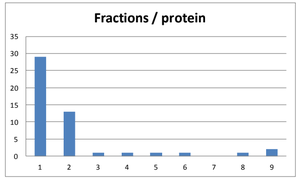


Fig. 11. Protein Distribution Across Slices. Most proteins were localized to 1 or 2 slices.

Table 2. Proteins vs Fractions11-15

Fraction 11				Fraction 12				Fraction 13				Fraction 14				Fraction 15			
Leng	Name	#Pep	#Obs	Leng	Name	#Pep	#Obs	Leng	Name	#Pep	#Obs	Leng	Name	#Pep	#Obs	Leng	Name	#Pep	#Obs
204	RPL15A	55	9	204	RPL15A	55	7	221	RPL10	55	6	151	RPS13	29	7	151	RPS13	29	3
254	RPL2A	50	8	254	RPL2A	50	9	199	RPL16A	39	8	143	RPS16A	25	6	143	RPS16A	25	7
255	RPS1A	89	7	255	RPS1A	41	6	255	RPS1A	39	6	142	RPL25	39	7	135	RPS24A	18	3
416	PGK1	58	4	416	PGK1	39	7	416	PGK1	44	6	172	RPL20B	18	4	136	RPL27B	25	3
256	RPL8B	42	9	225	RPS5	42	5	225	RPS5	47	8	136	RPL27A	31	5	138	RPL14A	26	8
332	TDH2	52	7	252	RPS0A	47	5	252	RPS0A	41	12	146	RPS18B	32	4	136	RPS17B	14	3
348	ADH1	82	4	256	RPL8A	32	4	247	GPM1	43	7	149	RPL28	26	5	119	RPS26B	31	3
458	TEF1	32	4	244	RPL7A	37	5	197	RPS9A	89	6	136	RPS17A	34	4				
252	RPS0B	41	7	199	RPL13B	89	6	172	RPL20A	32	3	190	RPS7A	32	5				
551	CDC14	77	5	240	RPS3	53	6	244	RPL7B	39	7	174	RPL11A	39	5				
613	SSB1	89	4					186	RPL18A	35	5	225	RPS5	25	4				
359	ERF2	57	4									138	RPL14B	31	3				
Ave			Ave				Ave				Ave				Ave				
384			261				238				159				137				

Each shared protein is marked with a distinct color.
No color : protein found in one fraction only.
Protein abbreviations correspond to standard gene symbols.

Conclusions

- Trypsin / capture membrane interfaces with SDS PAGE.
- Maintains spatial resolution.
- Produces terminal trypsin digestion peptides.
- Peptide recovery similar to in-gel digestion.
- PMF identifies up to ~ 10 proteins per slice.
- This workflow useful for quick characterization of protein preparations.

References

1) For more details, see Hattan, SJ, Du, J and Parker, KC (2015) Anal. Chem. 87:3685-93.

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