Development of Mass Spectrometry Cartridge for Det

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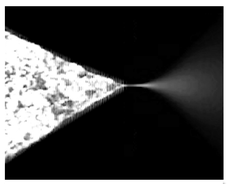
Overview

- A novel 3D printed cartridge performs rapid enzymatic digestion and peptide ionization from intact protein samples
- Online digestion of target protein performed by passing the protein solution through a pepsin immobilized membrane.
- Ionization was performed via electrospray from a carbon nanotube coated polymer substrate

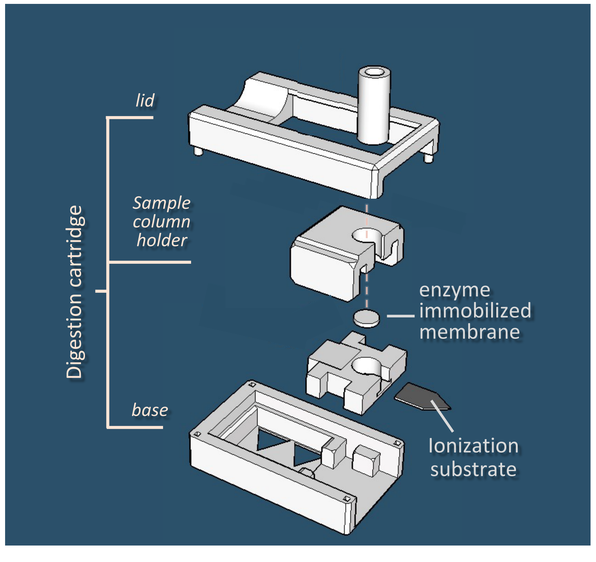
Introduction

- Clinical diagnostic tests of protein markers play a critical role in detection, diagnosis, and treatment of disease.
- Mass spec based assays could be routinely performed at or near the point of care, if its procedures could be dramatically simplified.
- In our previous work, an antibody-based enrichment step was built into a mass spec cartridge for the detection of target proteins from human plasma (*1*)
- We also previously developed an MS cartridge with built-in solid phase extraction (SPE) enrichment for small molecule detection (*2*).
- Here, we describe a cartridge which combines an enzyme immobilized membrane to perform rapid on-cartridge digestion of intact protein followed by immediate ionization using a built-in spray substrate .
- A 3D-printed mass spectrometry cartridge was developed for the on-cartridge digestion of proteins.



Picture of Tylor cone generated from a CNT-PE spray substrate

Methods

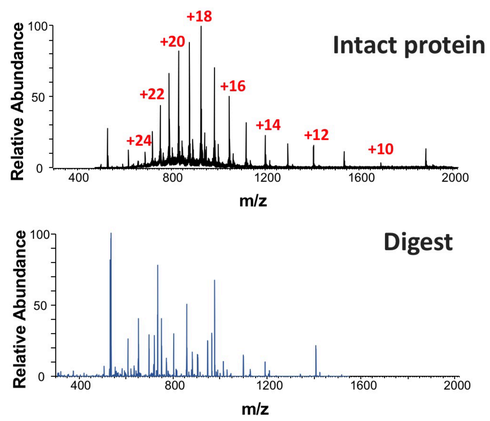


- A 3D printed cartridge was developed.
- The cartridge consists of a lid, a sample column, a column holder, and a base. All parts are assembled together. (LWH: 36mm x 22mm x 15mm)
- Pepsin was noncovalently immobilized onto a nylon membrane.
- Mass spectrometer: Thermo Scientific Q-Exactive Focus
- Ionization was performed by electrospraying from a carbon nanotube coated porous polyethylene (CNT-PE) spray tip (*1*).
- Digestion and ionization performed in 20:80 methanol:water with 5% formic acid

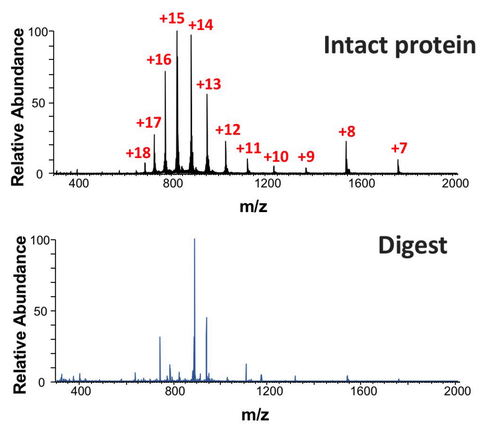
Results

Mass spectra of intact and digested proteins

Myoglobin, 10 µg/mL, MW: 16.7 kDa

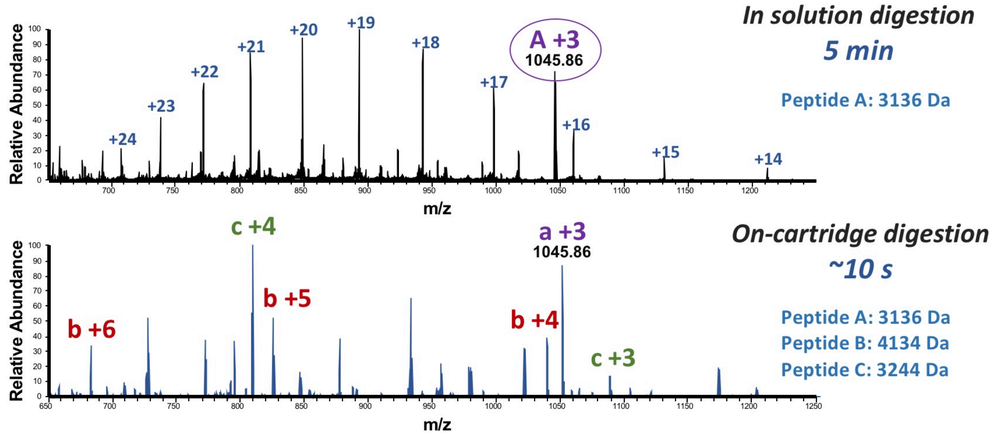


Cytochrome c, 50 µg/mL, MW: 12.4 kDa

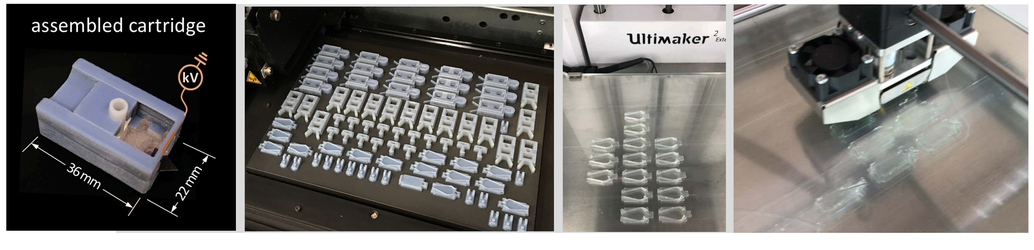


Rapid on-cartridge protein digestion

Proteolysis of myoglobin: On cartridge digestion 10 s > in-solution digestion for 5 min



3D printing cartridges



for Detection of Target Proteins Using On-cartridge Digestion

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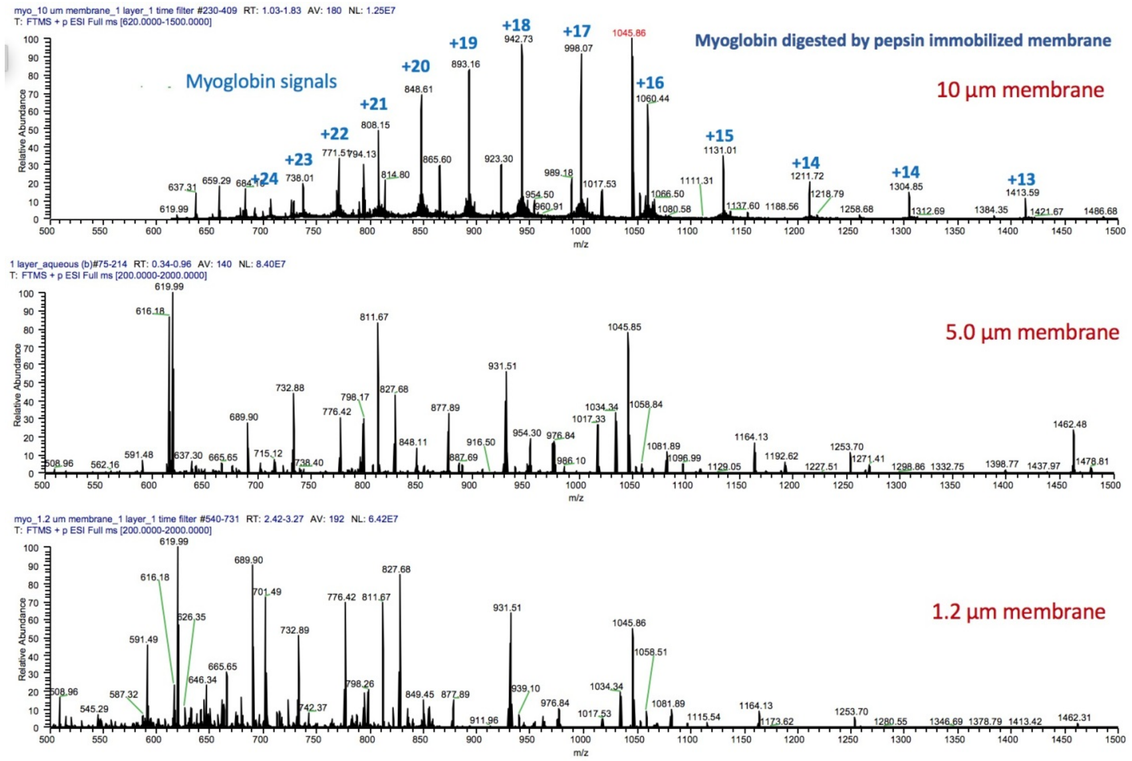
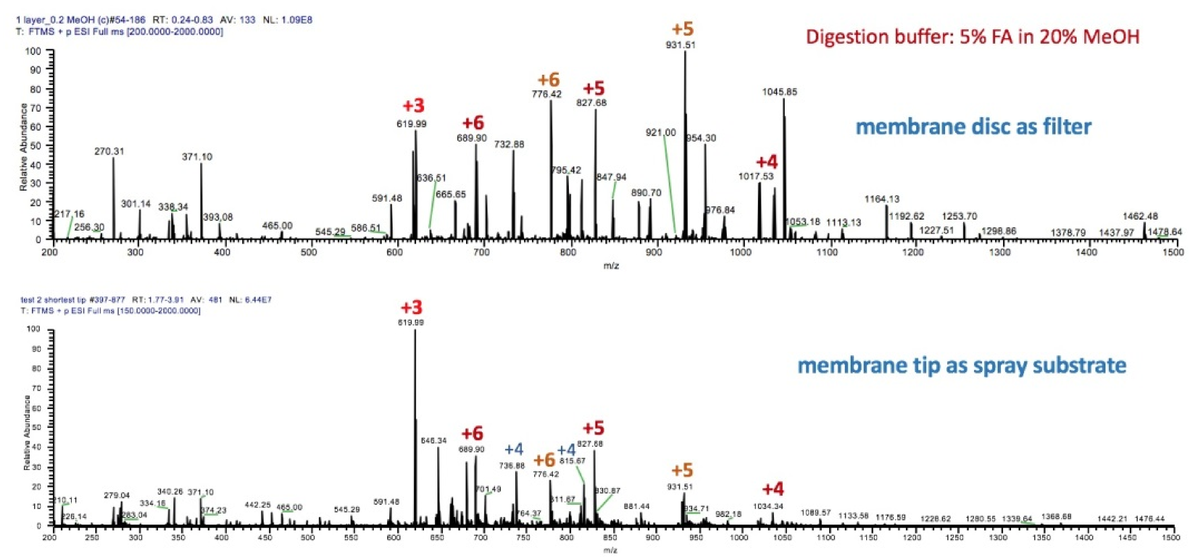
Effect of pore size on digestion



Digestion improves for smaller pore sizes. The 1.2 µm pore size is too small for the protein solution to pass through by gravity/capillary action. 5 µm gave adequate digestion while still allowing passive flow

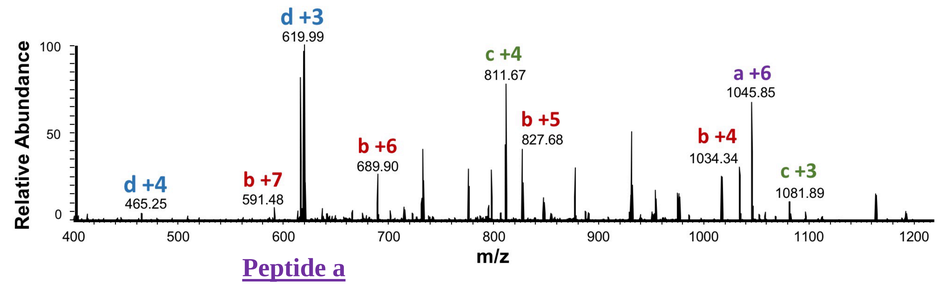
Spray directly from nylon digestion membrane



The nylon digestion membrane could be used directly as the spray substrate. The intensity of the most intense peptides was similar. The total number of peptides was lower compared to using a disc combined with the CNT coated PE spray substrate

Peptide identification

Four peptides were identified from equine heart myoglobin peptic digest using ExPASy. These peptides correspond to amino acids 2-30, 71-107, 108-138, and 139-154. A 73% sequence coverage was achieved in this preliminary on-cartridge digestion study.



Peptide a

MGLSDGEWQQ VLNVWGKVEA DIAGHGQEVL IRLFTGHPET LEKFDKFKHL KTEAEMKASE DLKKHGTVVL

Peptide b **Peptide c**

TALGGILKKK GHHEAELKPL AQSHATKHKI PIKYLEFISD AIIHVLHSKH PGDFGADAQG AMTKALELFR

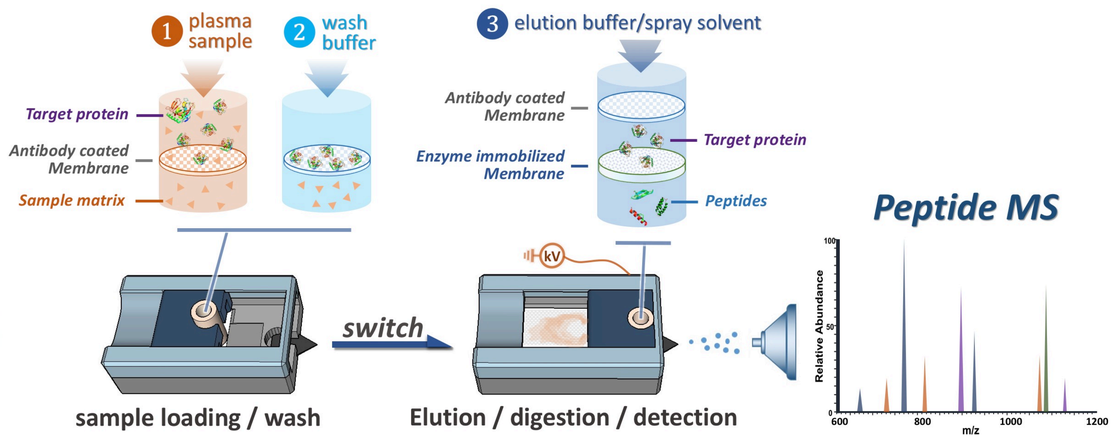
← Peptide d

NDIAAKYKEL GFQG

m/z errors typically < 1 ppm

Future Directions

- We will explore trypsin enzymatic digestion as well.
- We will combine the enzymatic digestion step described here with our previous work on antibody enrichment (*1*), depicted below



References

(1) Zhang, C.: Glaros, T.; Manicke N.E. *J. Am. Chem. Soc.*, **2017**, 139 (32), 10996–10999

(2) Zhang, C.; Manicke N.E. *Anal. Chem.*, **2015**, *87*, 6212–6219

We acknowledge support from Thermo