Multiplexed On-Target Protein Fractionation for MALDI Analysis of Sub-Proteome "Windows" from Complex Samples

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Overview

- Developed an on-target fractionation tool for MALDI-TOF analysis of complex protein samples
- The functionalized target surface displays a series of selective binding environments (>400 environments possible)
- Mouse heart muscle extract was used as the protein sample
- Different protein mass profiles were acquired from each binding environment
- New proteins were detected in both the bound and unbound fractions that were not present in the spectrum from the whole sample

Introduction

We have functionalized the surface of a MALDI target using molecular recognition-based building blocks to display a series of diverse binding environments. When incubated with a complex protein mixture, each environment captures a fraction of the proteins creating a series of unique "windows" into the proteome. Mouse heart muscle extract was incubated with each binding environment, the unbound protein solutions were removed and spotted in the standard way, and the binding environment was rinsed before matrix application. MALDI-TOF analysis was performed on each binding environment (bound fraction) and unbound protein solution (unbound fraction) using a Bruker Daltonics Ultraflex III MALDI-TOF/TOF Mass Spectrometer. The fractionation procedure takes approximately one hour and consumes only 5 µg protein per binding environment. The results below demonstrate that this method for multiplexed on-target protein fractionation creates sub-proteome "windows" into complex protein samples using a single on-target preparation procedure.

Results

- MALDI analysis of both bound and unbound fractions revealed new signals not identifiable in the whole sample
- Each binding environment behaved uniquely
- Some proteins were selectively bound, depleting them from the unbound fraction
- Some proteins were not bound by the binding environment and were therefore enriched in the unbound fraction
- Signals in the unbound fraction were sharper than in the whole sample which increased resolution making mass identification possible
- The total ion current increased for signals in both the bound and unbound fractions

Bound Fraction = Protein bound to the binding environment. MALDI was performed directly on the binding environment

Whole Sample = Unfractionated sample spotted directly on the target with standard matrix application

Unbound Fraction = Protein solution removed after incubation on binding environments and spotted with standard matrix application

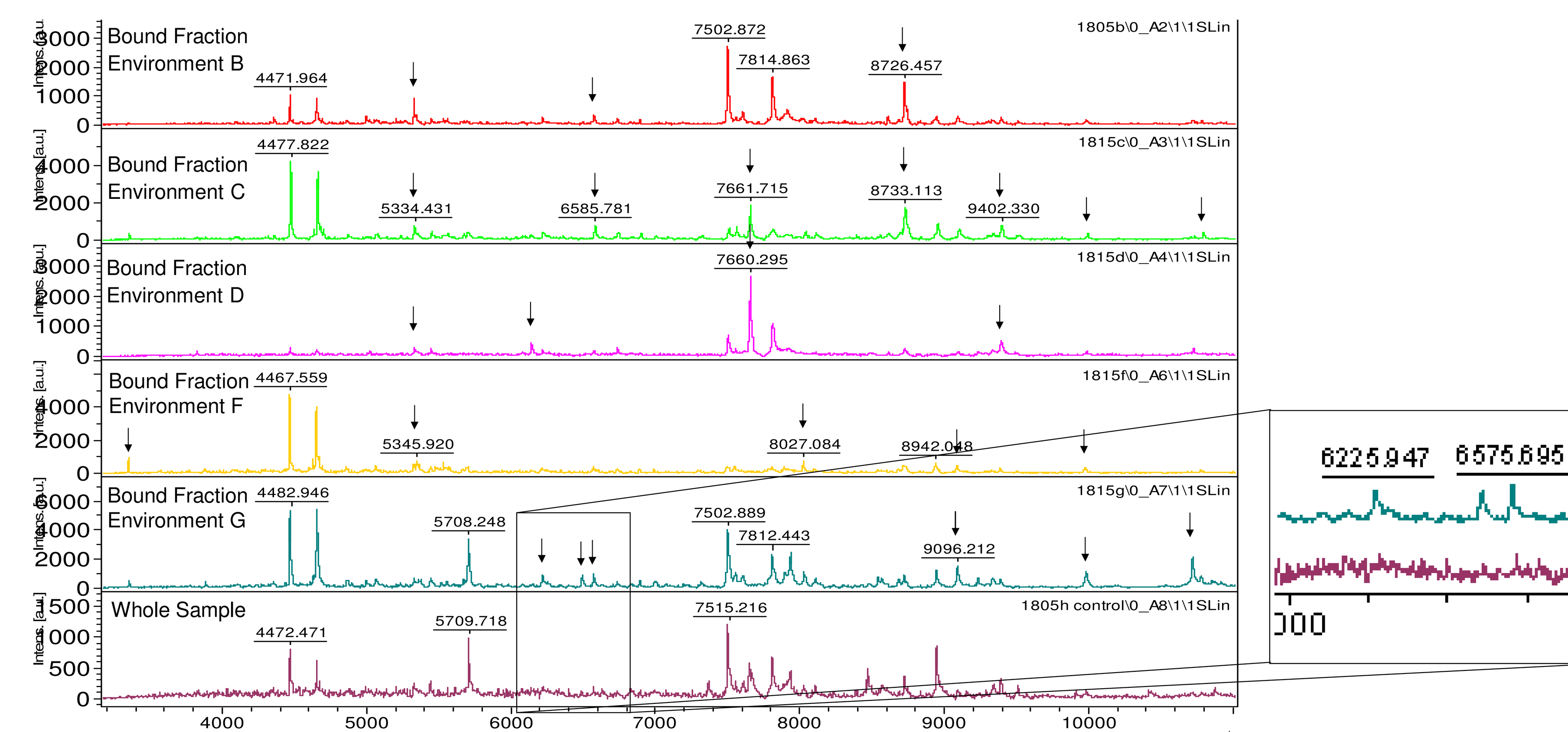

Figure 1. MALDI-TOF vs. the whole sample (bottom spectrum).
- New Peaks discovered after on-target fractionation (indicated with arrows)

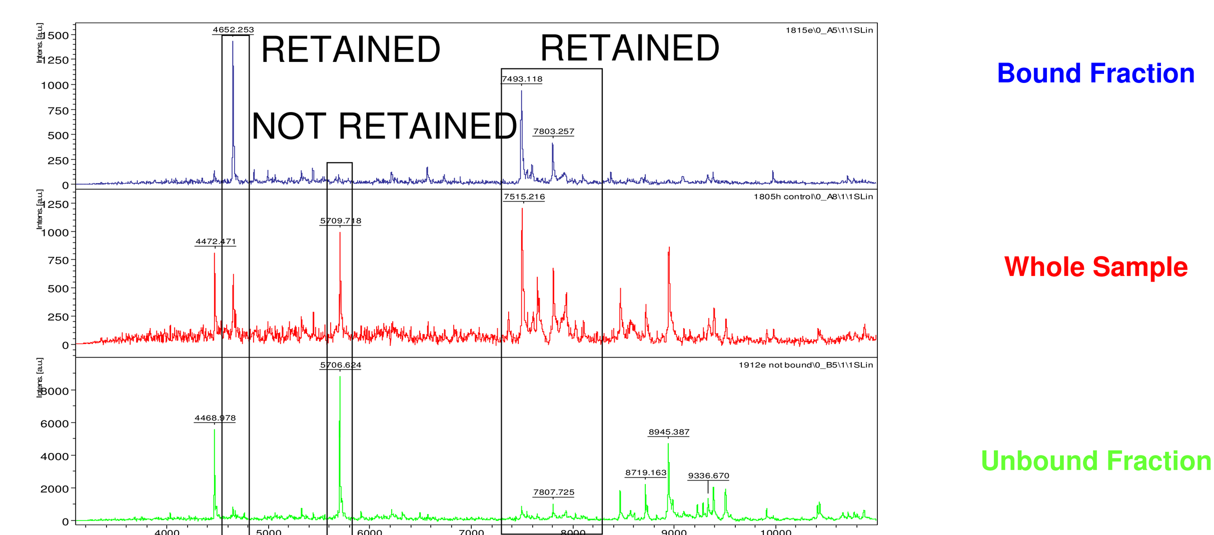

Figure 2. MALDI-TOF spectra of bound, whole and unbound muscle extract incubated on binding environment E.
- Selective retention of 4552 Da and 7493 Da proteins
- No retention of 5709 Da protein

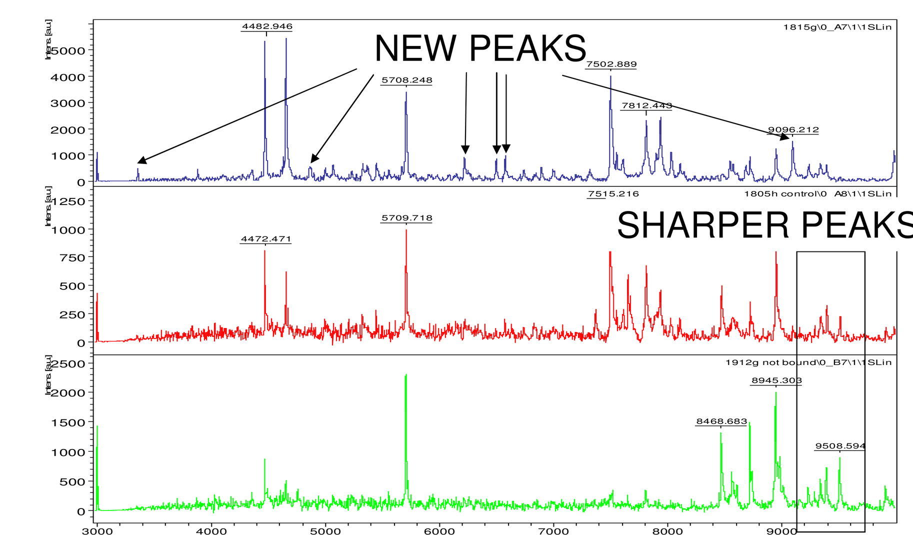

Figure 3. MALDI-TOF spectra of bound, whole and unbound muscle extract incubated on binding environment G.
- Increased resolution between 9200-9600 m/z in the unbound fraction
- Several new signals in the bound fraction

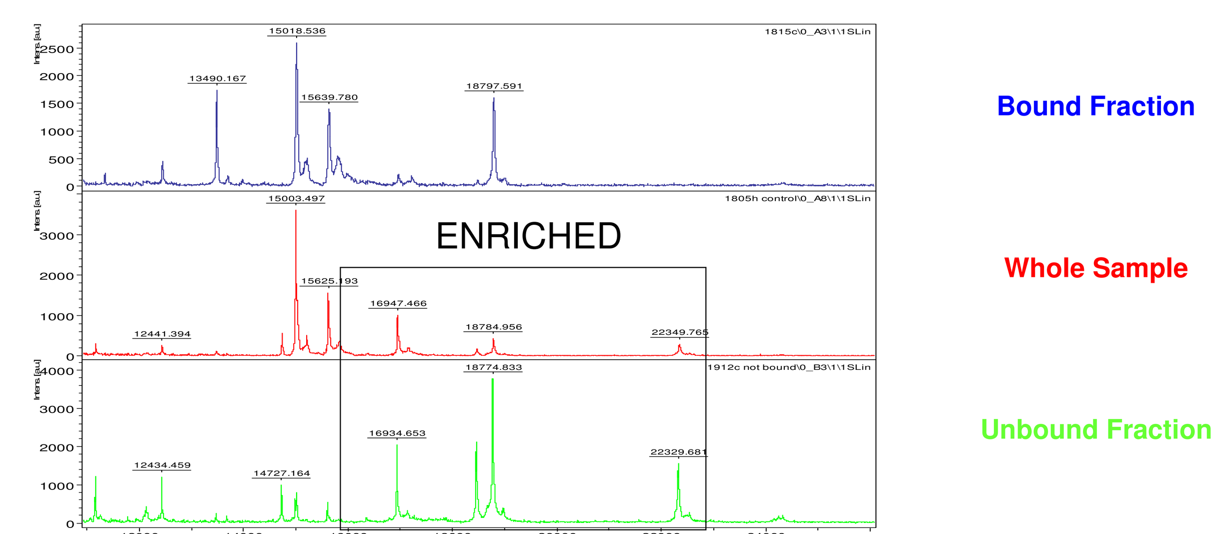

Figure 4. MALDI-TOF spectra of bound, whole and unbound muscle extract incubated on binding environment C.
- Several signals are enriched in the unbound fraction

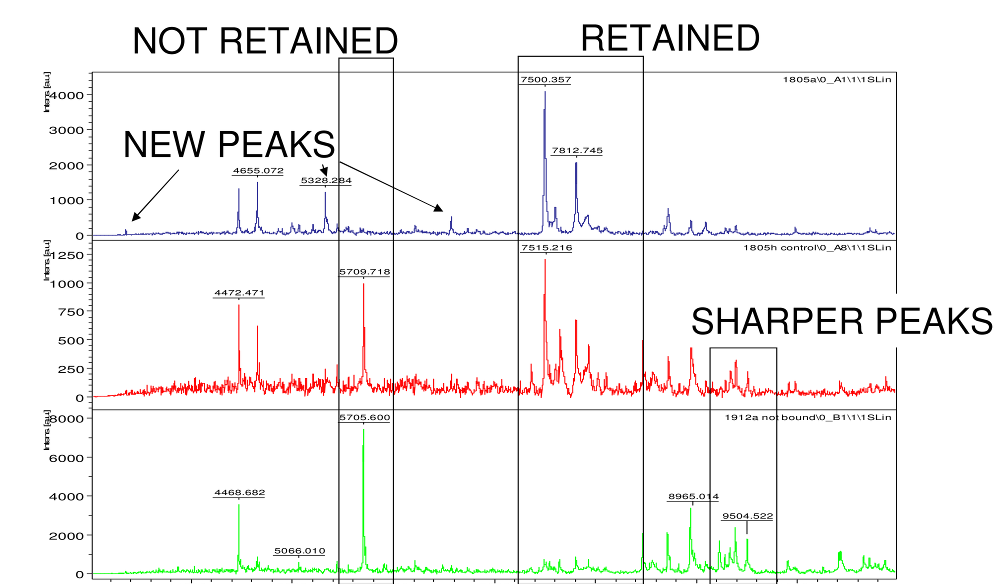

Figure 5. MALDI-TOF spectra of bound, whole and unbound muscle extract incubated on binding environment A.
- The signals between 9200 Da and 9600 Da are more clearly articulated in the unbound fraction
- The bound fraction revealed new signals

Methods

1. **Incubate sample on the target**

   Mouse heart muscle extract was prepared as described earlier.[1] The extract was concentrated in a 5 kDa cut-off centrifugal filter. 5 µL of the sample were added to each spot and incubated for 1 hr.

2. **Remove unbound fraction from the target**

   The unbound protein fraction was removed from the target surface and mixed with 5 µL of matrix solution (saturated solution of sinapinic acid in 50% Acetonitrile/water with 0.1% TFA). 1 µL was then spotted on a standard MALDI target spot.

3. **Wash Target**

   The target was washed by adding 5 µL of TBS to each spot and allowing it to sit for 10 min. This was repeated 3 more times. The next wash was 5 µL of 5 mM HEPES for 5 min followed by 5 µL water for 5 min.

4. **Add matrix**

   1 µL of the matrix solution was spotted on each environment and mixed by pipetting.

Conclusions

- Different binding environments selectively retained different proteins resulting in new protein profiles
- New peaks were observed in both the bound and unbound fractions
- The large number of potential binding environments, small amount of required sample, and efficiency of the procedure make this a powerful tool for biomarker discovery
- This tool can also be used for biomarker validation - the optimal binding environment can be selected using the above high-throughput procedure, then thousands of samples can be screened for the biomarker

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References

1.Hou, Y., Le Bihan, M. ,Vega-Avelaira, D., Coulton, G.R., *Proteomics* 2006, 6, 3096-3108.