



Advances in Capillary Liquid Chromatography for High-Throughput Top Down Proteomics

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OVERVIEW

- A separation platform utilizing a modified GELFrEE method coupled to capillary liquid chromatography reduces protein sample complexity analysis.
- Mass spectrometry data acquisition using ion trap precursor scans followed by nozzle-skimmer dissociation and FT fragmentation scans affords identification of proteins greater than 70 kDa on an LC timescale.
- A newly designed capillary column heater allows for decreased retention time and peak width.
- Modifications to the mobile phases show promise for further increasing protein separation.

INTRODUCTION

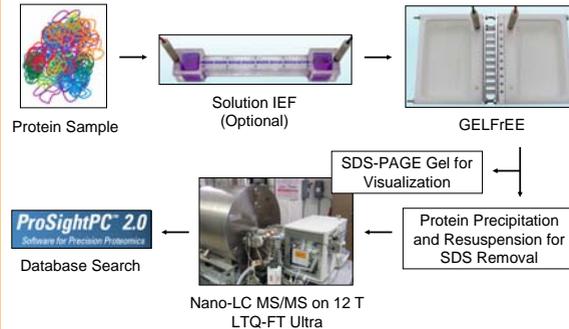
Top Down proteomics has benefited greatly from advances in mass spectrometry instrumentation and database searching, yet it has been hindered by the lack of robust separation platforms for intact proteins. Recently, the use of Gel-Eluted Liquid Fraction Entrapment Electrophoresis (GELFrEE)^{1,2} followed by capillary liquid chromatography-MS/MS has enabled hundreds of Top Down identifications from a single proteome run.³ However, most of the identifications occur in the low-molecular weight proteome (<40 kDa). Proteins of higher molecular weight, which comprise a majority of the human proteome, have been difficult to identify in great numbers⁴ largely due to poor chromatographic performance. In this work, we utilize column heating and modifications to the mobile phase to increase the peak capacity of LC separations.

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METHODS

- All buffers and gels were prepared according to Laemmli protocol. The separation potential was 180 V. The gel tube consisted of a 1 cm 4% gel and a 3 cm 8% resolving gel. 150 μ L fractions were collected and 10 μ L were used for SDS-PAGE visualization.
- SDS was removed from the GELFrEE fractions using MeOH/CHCl₃/H₂O precipitation and resuspended in 30 to 40 μ L of H₂O with 5% ACN and 0.2% formic acid.
- Capillary-LC separations were performed using a 10 cm long, 75 μ m inner diameter columns packed with 5- μ m PLRPS particles with 1000 Å pore size.
- Typical capillary LC solvents were A: H₂O with 0.2% formic acid and B: 95% ACN, 5% H₂O with 0.2% formic acid. In some experiments TFA was added at 0.05% or 0.1% or IPA with 0.2% formic acid was used as solvent B.
- Fragmentation was performed using nozzle-skimmer dissociation set to 75V.

ANALYSIS PLATFORM



HIGH RESOLUTION GELFrEE

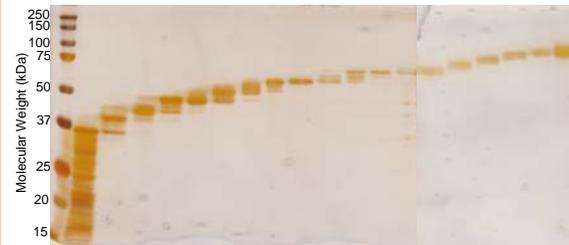
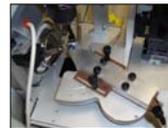


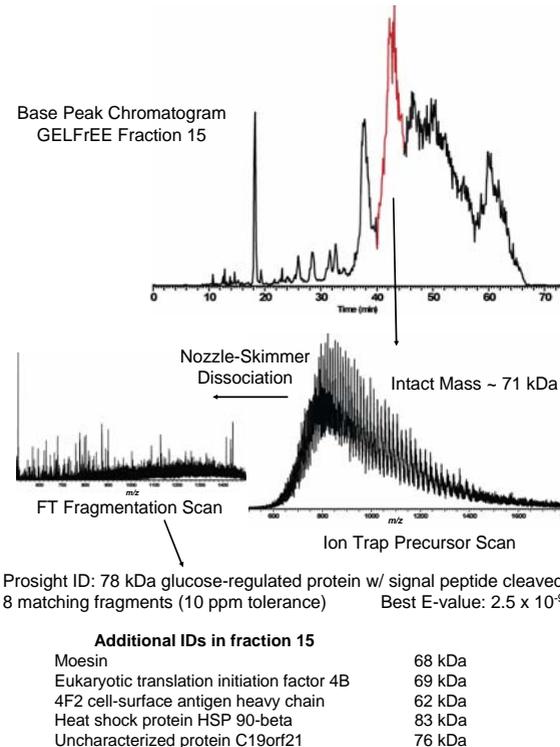
Figure 1: SDS-PAGE (12% T) visualization of the GELFrEE separation of 400 μ g of HeLa cytosolic proteins. The visualization shows the ability of the GELFrEE platform to fractionate higher molecular weight proteins into narrow size bins.

CAPILLARY COLUMN HEATER

- Easily couples to New Objective's PicoView[®] nanospray source via a magnetic stage.
- Allows for entire packed bed of an analytical column to be heated, spraying sample from the pulled tip extending from the heater block.
- Conductive transfer block allows for fast temperature stabilization and has been used to heat the column to 60°C.



PROTEIN DETECTION AND IDENTIFICATION



USE OF TRIFLUOROACETIC ACID

- Use of TFA as an ion pairing agent has shown significant decreases in peak width across the molecular weight range.
- Signal suppression has been observed using as low as 0.05%, leading to a decrease in identifications.

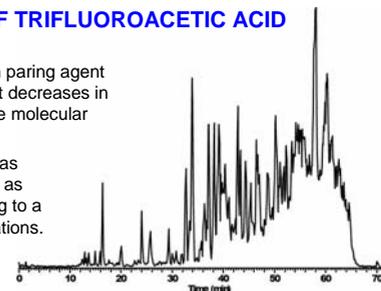


Figure 2: Base peak chromatogram from GELFrEE fraction 2 using 0.1% TFA.

CAPILLARY COLUMN HEATING

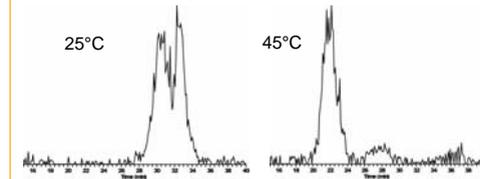


Figure 3: Selected ion chromatogram using two high-resolution fragments of glyceraldehyde-3-phosphate dehydrogenase (36 kDa) identified in an IEF-GELFrEE fraction. The peak width and retention time decrease when the capillary temperature is increase to 45°C. The separation used isopropanol as solvent B.

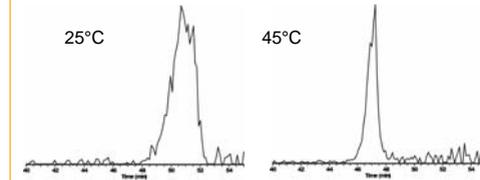


Figure 4: Selected ion chromatogram using two high-resolution fragments of an identified 65 kDa protein from a high-resolution GELFrEE fraction. Again, the peak width and retention time both decrease significantly when the capillary temperature is increase to 45°C. The separation used 0.1% TFA.

CONCLUSIONS

- High resolution GELFrEE allows for the separation of a complicated proteome into narrow size bins for Top Down analysis of higher molecular weight proteins.
- A newly designed capillary column heater allows for heating the chromatographic separation, decreasing retention time and peak width.
- TFA has shown to decrease peak width but has led to a decrease in sensitivity.

FUTURE WORK

- Future work will be focused on determining the concentration of TFA or other ion pairing agents such as hexafluoroisopropanol that consistently allow for increased peak capacity as well as an increase in protein identifications.
- The use of isopropanol will be further explored for increasing resolution and sensitivity of high molecular weight proteins.
- Hydrophilic interaction liquid chromatography (HILIC) will be coupled to the mass spectrometer for the analysis of GELFrEE fractions.

ACKNOWLEDGEMENTS

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