# Top Down Proteomics Using Online Polymer Reversed Phase (PLRP) Nanocapillary-LC Coupled Fourier Transform Mass Spectrometry

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## OVERVIEW

In comparison to Bottom Up proteomics, the growing field of Top Down proteomics is impeded for high throughput, due partly to the lack of comparable studies in sample preparation and online separation methods.

Here, we evaluate the performance of Polymer Reversed Phase (PLRP) for online nano-LC coupled Top Down analysis in combination with pre-fractionation by gel-eluted liquid fraction entrapment electrophoresis (GELFrEE).

## INTRODUCTION

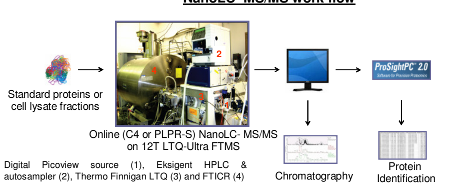
Due to its uniformly hydrophobic nature, enhanced chemical, mechanical, and pH stability, the polymeric (polystyrene divinylbenzene) reversed phase resin is well suited for biopolymer separation. The silica based reversed phase hydrophobic separation media such as C4, C5, and C18 have been widely used for nano-LC based separation of peptides, and proteins. Suitability of capillary packed PLRP for online nano-LC separation of intact proteins in combination with ultra high resolution Fourier Transform mass spectrometry (FTMS) has yet to be studied in detail.

For reducing the complexity of eukaryotic proteomes such as yeast and human, we have recently adopted a separation device known as gel-eluted liquid fraction entrapment electrophoresis (GELFrEE). GELFrEE separates intact proteins according to mass into well resolved liquid fractions.

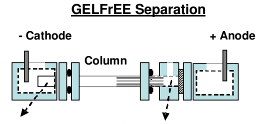
The advantage of PLRP nano columns over the conventional C4 nano columns, and its application to high throughput online identification and characterization of high mass proteins using 12T LTQ-Ultra FTMS is discussed.

## METHODS

**NanoLC- MS/MS work flow**



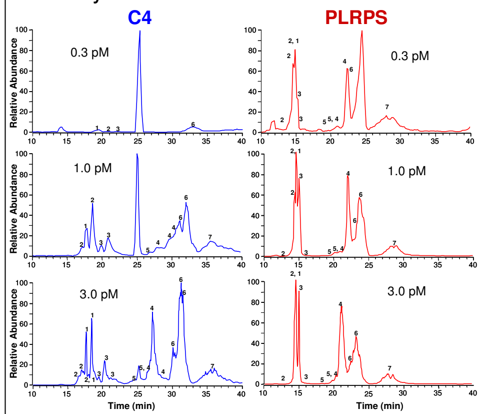

**GELFrEE Separation**



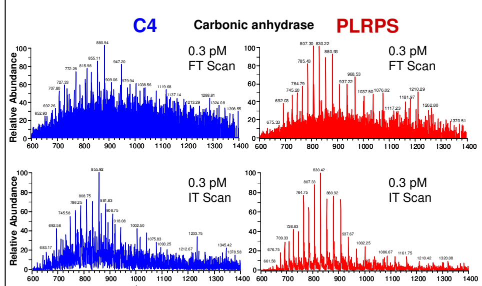

Whole cell lysate resuspended in Laemmli buffer was introduced into the GELFrEE device for size separation. GELFrEE device was operated as described previously (Tran, J.C., and Doucette, A.A. (2008). Anal. Chem. 80(5): 1568-1573).

## RESULTS

### Recovery and Resolution

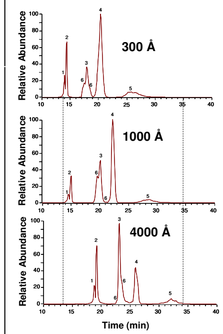



Chromatograms of RPLC separation of a seven protein standards mixture with varying concentrations, using C4 (blue) and PRLPS (red) (75 µm I. D. x 100 mm, 300 Å, 5 µm stationary phase) analytical columns. Standards used were: 1. Ubiquitin; 2. Cytochrome c; 3. α-lactalbumin; 4. Myoglobin; 5. α-casein; 6. Carbonic Anhydrase; 7. Ovalbumin. PLRPS provides reduced peak widths and higher recovery leading to increased chromatographic resolution and MS sensitivity.



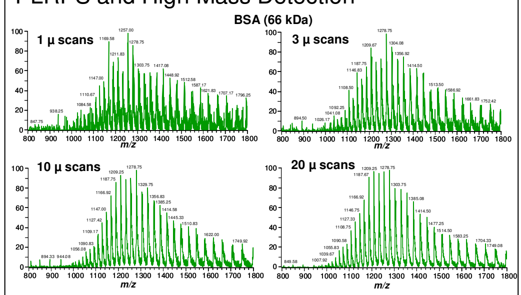

Mass spectra for carbonic anhydrase obtained through online C4 (blue) and PLRPS (red) RPLC separation. The mass spectra show that PLRPS exhibit increased S/N in MS detection resulting from increased recovery.

### PLRPS Pore Size and Retention



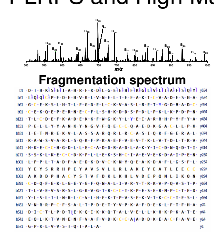

Separation of six protein standards ranging from low mass to medium- high mass were conducted on nano-LC using varying pore size. Chromatograms show inverse relationship between pore size and retention factor. These figures show that 1000 Å is the optimal pore size for both medium (30-45kda) and high MW proteins (>50kDa) in terms of resolution. In addition, recovery was observed to be inversely proportional to pore size. Protein standards used were: Cytochrome C; 2. α-lactalbumin; 3. Myoglobin; 4. Carbonic anhydrase; 5. Ovalbumin; 6. Bovine serum albumin.

### PLRPS and High Mass Detection




Ion trap charge state distribution profiles for BSA fractionated online using PLRPS (300 Å) from six protein standard mixture with increase in number of micro scans. The S/N intensity increases with micro scans.

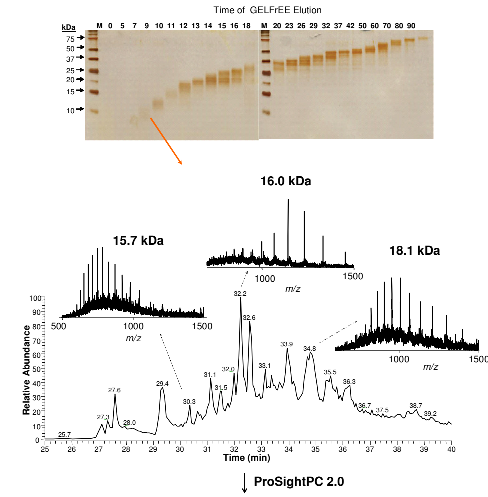
### PLRPS and High Mass Identification




Bovine serum albumin (Uniprot ID: P02769)

Shown here is a fragmentation spectrum from single FT scan obtained during the online nano-LC MS/MS of six protein standards mixture. Monoisotopic fragment masses were obtained from this scan using Xtract and searched against a standard protein database consisting of 7365 uniprot protein forms. The search result showed 23 fragments matching to BSA with mass error of less than 5 ppm.

### GELFrEE Separation, PLRPS NanoLC-MS/MS




| Category | Search Type | Matching Fragments | Theor. Mass | Protein Description | Intact ID | Accession Number | PTMs | P Score | E Value |
|---|---|---|---|---|---|---|---|---|---|
| good | absolute_mass | 17 | 11662.8 | >B8ZZQ6_HUMAN, Putative uncharacterized protei | 1 | B8ZZQ6 | N-acetyl-L-serine (1) | 2.32E-27 | 8.76E-22 |
| good | absolute_mass | 14 | 10521.6 | >HMGN1_HUMAN,Non-histone chromosomal prote | 1 | P05114 | | 5.93E-26 | 2.24E-20 |
| good | absolute_mass | 14 | 11950.8 | >Q15204_HUMAN,Full=Prothymosin alpha;. | 1 | Q15204 | N-acetyl-L-serine (1) | 3.21E-22 | 1.21E-16 |
| good | absolute_mass | 11 | 19502 | >B4DN70_HUMAN, cDNA FLJ51902, highly similar | 1 | B4DN70 | | 4.06E-18 | 1.53E-12 |
| good | absolute_mass | 12 | 9256.02 | >HMGN2_HUMAN,Non-histone chromosomal prote | 1 | P05204 | | 1.33E-16 | 5.01E-11 |
| good | absolute_mass | 10 | 6717.97 | >B8ZZI3_HUMAN, Putative uncharacterized protein | 1 | B8ZZI3 | | 2.4E-16 | 9.04E-11 |
| good | absolute_mass | 8 | 7046.58 | >MT2_HUMAN,Metallothionein-2; Short=MT-2; AltN | 1 | P02795 | | 1.75E-12 | 6.58E-07 |
| good | absolute_mass | 7 | 15093.6 | >B7Z5A2_HUMAN, cDNA FLJ57759, moderately sir | 1 | B7Z5A2 | N-acetyl-L-alanine (1) | 1.75E-12 | 6.59E-07 |
| good | absolute_mass | 8 | 17172.2 | >B7Z6S5_HUMAN,cDNA FLJ50699, moderately sim | 1 | B7Z6S5 | N-acetyl-L-alanine (1) | 2.67E-12 | 1.01E-06 |
| good | absolute_mass | 7 | 22235.3 | >B5BU26_HUMAN, Peroxiredoxin 1 (Peroxiredoxin | 1 | B5BU26 | N-acetyl-L-serine (1) | 2.28E-11 | 8.59E-06 |
| good | absolute_mass | 7 | 15153.8 | >A6NIW5_HUMAN,Peroxiredoxin 2, isoform CRA_a | 1 | A6NIW5 | N-acetyl-L-alanine (1) | 3.87E-11 | 1.46E-05 |
| good | absolute_mass | 7 | 16060.5 | >B7ZB63_HUMAN,cDNA, FLJ79427, highly similar t | 1 | B7ZB63 | | 6.1E-11 | 0.000023 |
| good | absolute_mass | 7 | 12173.7 | >B9ZVP7_HUMAN,Putative uncharacterized protein | 1 | B9ZVP7 | N-acetyl-L-serine (1) | 6.85E-11 | 2.58E-05 |
| good | absolute_mass | 6 | 11229.6 | >Q8TE09_HUMAN,SLTP001;. | 1 | Q8TE09 | N-acetyl-L-alanine (1) | 9.55E-11 | 0.000036 |
| good | absolute_mass | 7 | 17871.3 | >B4DJC8_HUMAN, cDNA FLJ51215, highly similar | 1 | B4DJC8 | | 1.15E-10 | 4.34E-05 |
| good | absolute_mass | 7 | 47350.4 | >ENOA_HUMAN, Alpha-enolase; EC=4.2.1.11; AltN | 1 | P06733 | | 1.17E-10 | 4.42E-05 |
| good | absolute_mass | 7 | 17097 | >Q9H7Z5_HUMAN, cDNA FLJ14048 fis, clone HEM | 1 | Q9H7Z5 | N-acetyl-L-threonine (1) | 5.88E-10 | 0.000222 |
| good | absolute_mass | 6 | 16901.4 | >IF5AL_HUMAN, Eukaryotic translation initiation fac | 1 | Q6IS14 | N-acetyl-L-alanine (1) | 8.02E-10 | 0.000303 |
| good | absolute_mass | 6 | 38861.3 | >B4DW52_HUMAN,cDNA FLJ55253, highly similar | 1 | B4DW52 | N-acetyl-L-aspartic acid (1) | 1.17E-09 | 0.00044 |
| good | absolute_mass | 6 | 18991.6 | >Q5T9W8_HUMAN, Heat shock protein 90kDa alph | 1 | Q5T9W8 | | 1.86E-09 | 0.0007 |
| good | absolute_mass | 6 | 8649.39 | >Q6E433_HUMAN, Activated RNA polymerase II tra | 1 | Q6E433 | | 2.51E-09 | 0.000945 |

Silver stained gel image of fractions collected from GELFrEE separation of HeLa cell lysates and undergone SDS removal procedure (top). Selected fraction (highlighted in red) was injected to nanoLC-MS/MS analysis. Through ProSightPC database search in absolute search mode against human database, the single injection afforded 22 unique protein identifications.

## CONCLUSIONS

- PLRPS provides improved resolution and recovery over C4
- 1000 Å was the optimal pore size for resolution
- Coupling GELFrEE with PLRPS enables nanoLC-MS/MS analysis of complex proteome mixtures
- The standardized methods will be applied for the identification and characterization of proteins from other proteomes such as the secretome of human glioblastoma cell lines.

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