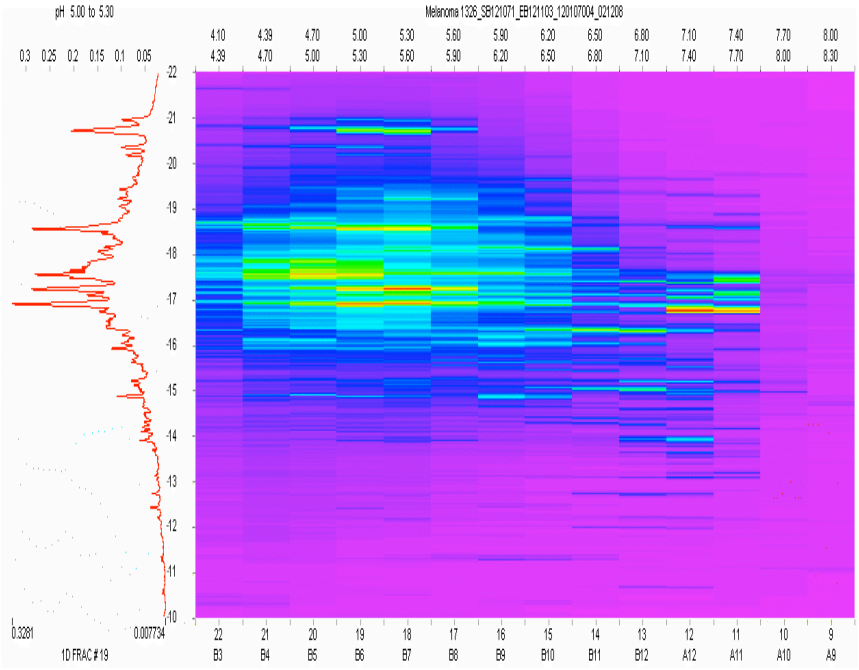
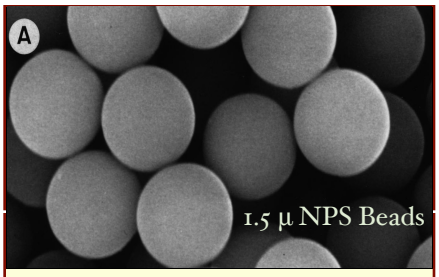
ProteoSep HPRP columns as a fast, high resolution alternative to 1D SDS-PAGE

With the advent of non-porous silica (NPS) based HPLC columns[1] it is now possible to perform reproducible, high resolution separations of highly complex protein and peptide mixtures containing surfactants, chaotropes, salts, etc., without the problem of fouling ubiquitous to porous silica based columns. Moreover, NPS-RP HPLC columns [ProteoSep HPRP] allow for significant automation and speed with all proteins retained "Intact" and in the liquid phase for easy isolation and transfer to other analytical techniques like MS and ELISA based analyses. This capability has been the hallmark of success for the ProteoSep 2D fractionation technology[2-4].

Illustrated below is the automated ProteoSep HPRP analysis of 16 different pH fractions collected for a primary Melanoma tumor cell lysate using a ProteoSep Chromatofocusing (HPCF) column imaged using ProteoVue Software.

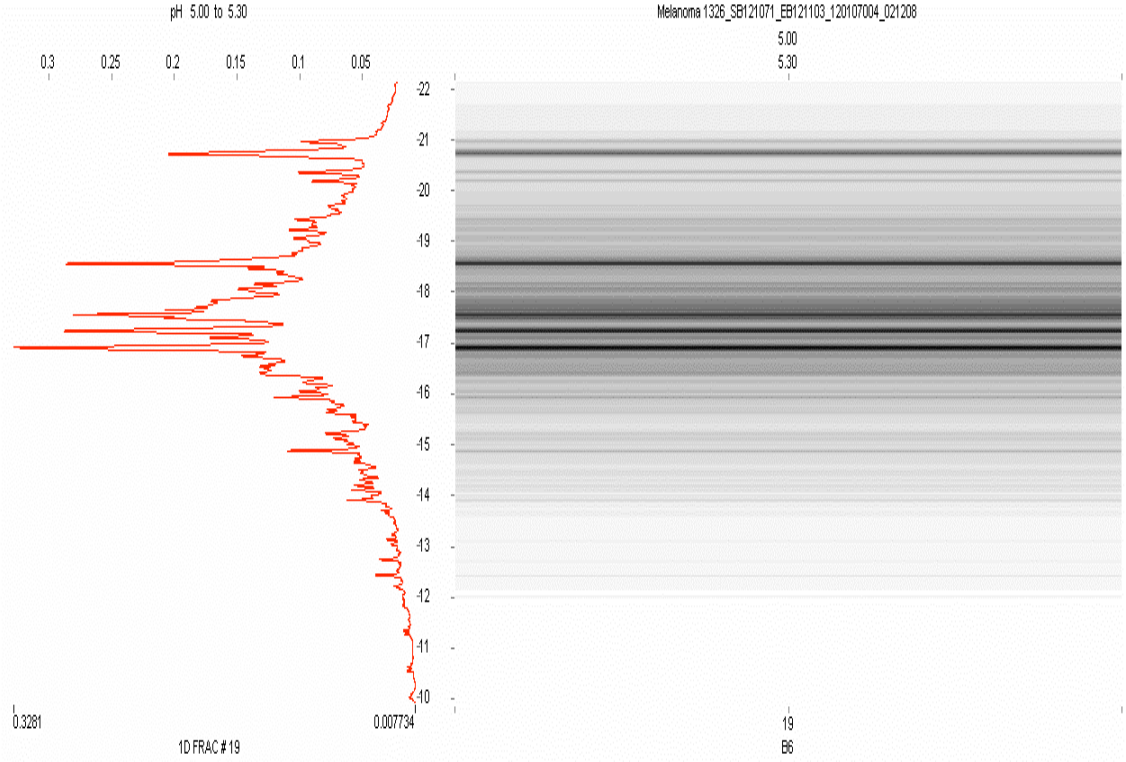




NPS® – An Ultra-Fast, high-resolution HPLC support tailored specifically for Peptides, Proteins & MS. The totally non-porous nature of NPS provides for fast mass transfer kinetics and high recovery of proteins at low surface carbon loads and organic MP modifiers. This allows for easy elution and recovery of highly hydrophobic proteins.

In essence, each pH fraction analysis is a "1D Hydrophobicity Analysis" of the proteins having a defined pI range. For the pH fraction 5.0-5.3 highlighted, there are >70 distinct protein bands identified by the ProteoVue analysis and imaging software. All salts, chaotropes, ampholines and additives present in the buffers used for the pI fractionation are removed in the 1st 2-5 minutes of the standard HPRP analysis gradient.

1D HPRP Analysis" Chromatogram and ProteoVue image for proteins with pI's of 5.0-5.3 present in a primary melanoma patient tumor cell extract



By collecting fractions over short time periods of the protein elution profile (typically 0.25 minutes/well), it is easy to see that discrete bands can be isolated (into 96 well plates) in the liquid phase for ready analysis using MS or antibody detection techniques, as well as CE or labchip electrophoresis methods. ProteoSep HPRP analysis uses only Acetonitrile-Water-TFA gradient solvent elution providing for easy solvent removal from the isolated proteins or peptides.

In many complex biological samples, however, 2D resolution of the proteins or peptides is not always necessary to follow-on analysis using ELISA or MS based methods. High Resolution analysis of very complex protein and peptide samples is equally possible for many important sample types such as cell and tissue lysates, sub-cellular fractionations, membrane proteins, all types of biofluids, tryptic digests, etc. where a more rapid surveying of potential protein changes is desired.

1D Proteomics Strategies using *NPS* RP HPLC[5]

This 1D fractionation and analysis technique has some significant benefits over complex protein analysis using 1D SDS-PAGE analysis.

1. **Significantly easier set-up and operation with the ability to automate using standard HPLC Instrumentation – No complex column regeneration protocols necessary.**
   - **Several hundred 1D analyses on a single ProteoSep HPRP column possible.**
   - **Significantly higher resolution than SDS-PAGE with <60 min analysis times.**
2. **No need for staining to image the proteins with comparable or better LODs.**
3. **Automated collection of the intact proteins from liquid fractions into multi-well plates without resorting to protein digestion.**
4. **Scaleable to 100's ug loadings for semi-preparative work.**
5. **Analysis and imaging software for quantitative analysis of proteins.**
6. **Run Single Analyses without wasting a whole gel.**
7. **Multi-year lifetimes HPRP columns eliminates pre-packed Gel storage issues.**
8. **The ability to use HPLC gradient modification to increase the resolution of proteins & peptides for complex expression systems (see example below).**
9.

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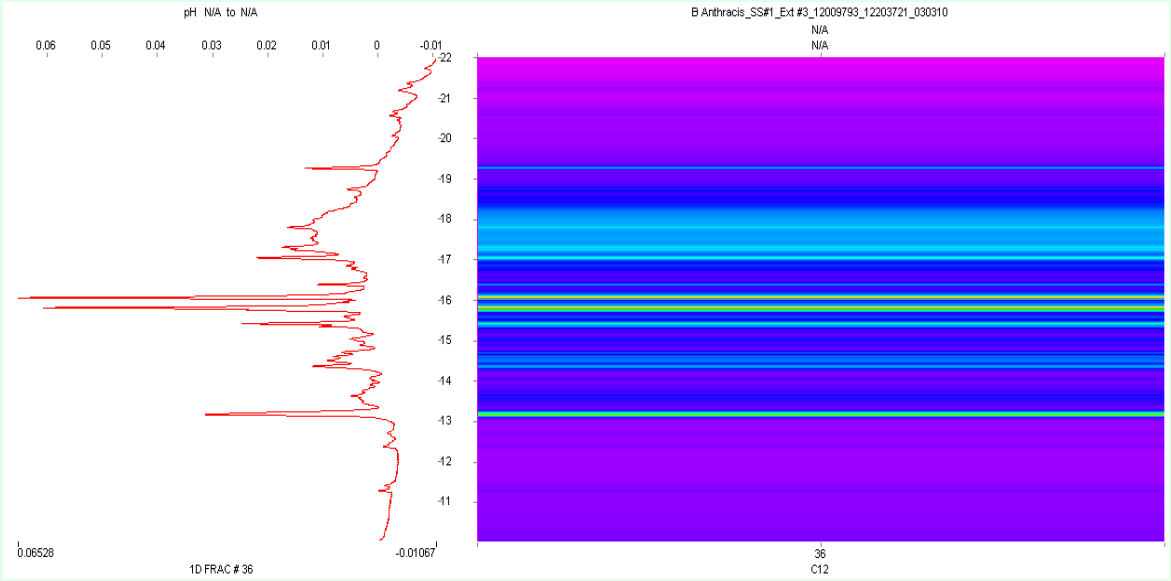
These 2 figures illustrate the results of a 1D ProteoSep HPRP analysis of the surface coat proteins obtained from a B. Anthracis spore extract using 2 different HPRP solvent gradient profiles

The "**Standard**" Linear Acetonitrile-Water-TFA Gradient provides for resolution of 35 protein bands over a 30 minute gradient time.

"Standard" Linear Gradient:
A – 0.1% TFA/Water
B – 0.08% TFA/ACN
0-100 % B in 30 min

48 fractions collected for analysis

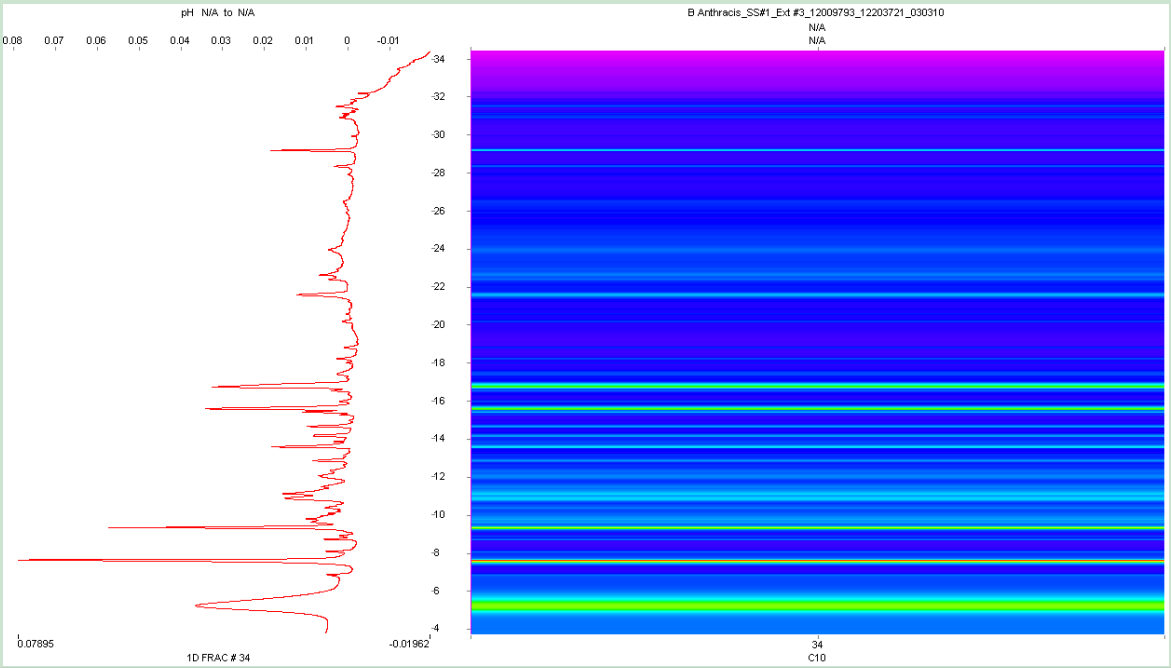


Using the **Expanded** Range non-linear Gradient improves the resolution to > 60 protein bands over the same 30 minute gradient time!

"Optimized" Non-Linear Gradient:
A – 0.1% TFA/Water
B – 0.08% TFA/ACN

96 fractions collected for analysis

0-5% B in 2 min, 5-15% B in 1 min, 15-25% B in 2 min, 25-30% B in 3 min, 30-41%B in 15 min, 41-47% B in 4 min, 47-67% B in 5 min, 67-100% B in 3 min



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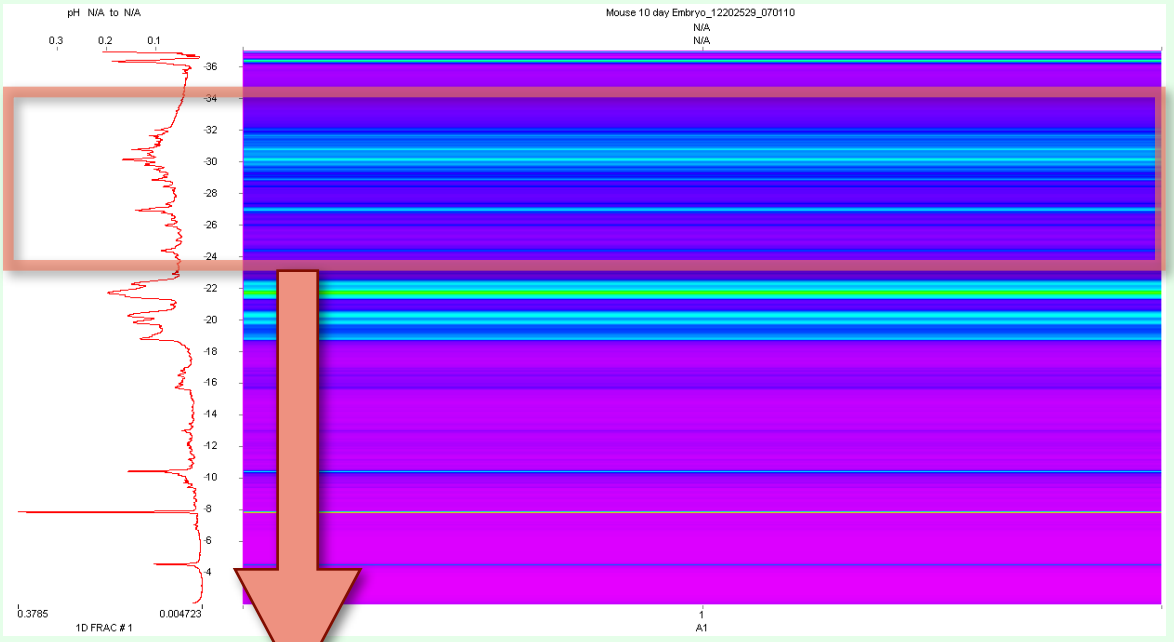
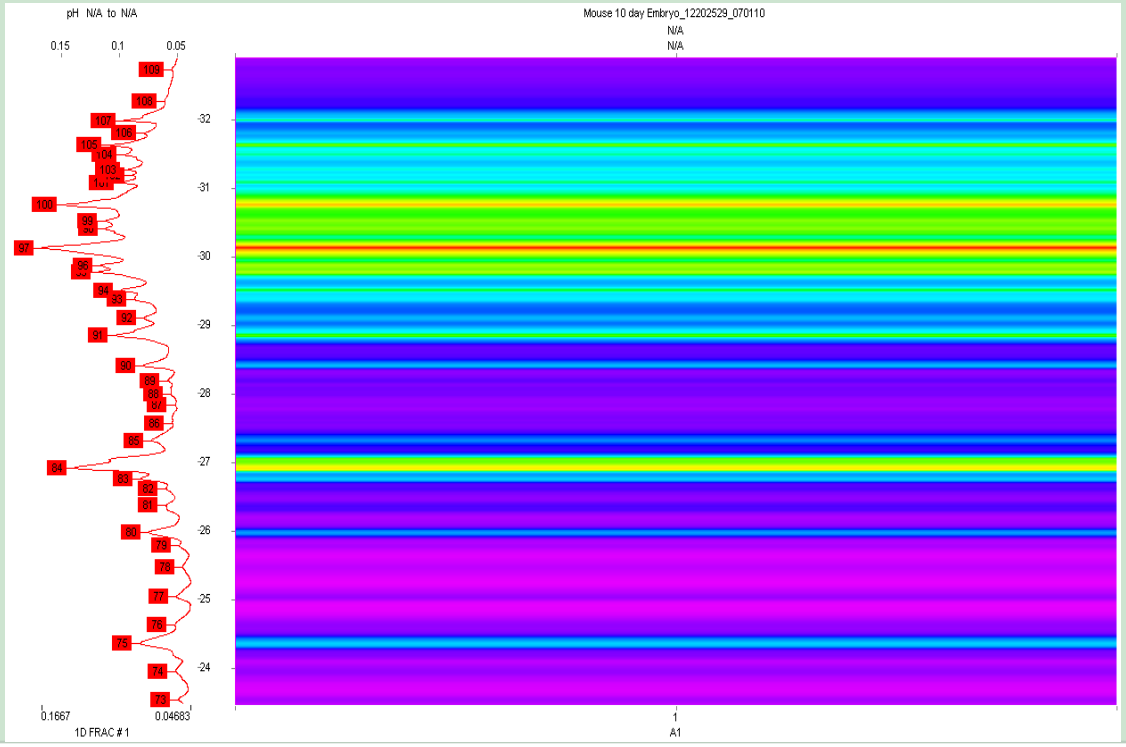
1D ProteoSep HPRP analysis of 10 day old Mouse embryos using the optimized non-linear gradient – >125 distinct protein bands identified!



Expanded region outlined in the area above – 36 protein bands identified (red boxes).



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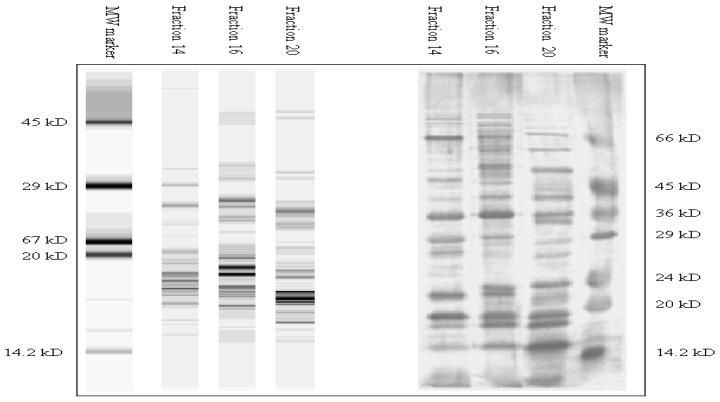
It is important to note that the separation of proteins by ProteoSep HPRP columns is based on protein Hydrophobicity whereas 1D SDS-PAGE analysis is by MW. Below is the comparative analysis of some pH fractions form a human erythrolukemia (HEL) cell lysate showing the direct comparison of the two techniques. As expected protein Hydrophobicity is not equivalent to MW.

For 1D Gel analysis, however this information is considered to be "low-value" information since the MW resolution is typically low and PTM effects can confound any MW comparative analysis for routine 1D gel separations. ["Protein Electrophoresis: The Benchside View": Garone, L., Research Review in Genomics and Proteomics, March 2006]

HEL Cell Lysate Fractions



NPS RP-HPLC 1D Gel

Wall, DB; et.al., Anal. Chem. 72(6) 2000, 1099-1111

1. Fast Chromatography and Nonporous Silica", Barder, TJ, et. al., LC-GC **15**(10) 918-926, 1997
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5. "All-Liquid Separations, Protein Microarrays and Mass Spectrometry to Interrogate Serum Proteomes: an Application to Serum Glycoproteomics", Patwa, TH, et.al, Methods Mol. Biol. **520**, 75-87, 2009 and references therein