IS SELECTIVE AFFINITY DEPLETION OF ABUNDANT SERUM PROTEINS USEFUL AND REPRODUCIBLE?

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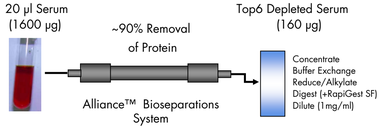
OVERVIEW

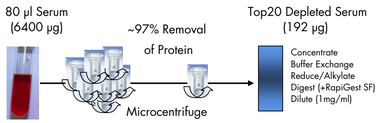
- Selective affinity subtraction of abundant proteins from serum and plasma is an attractive solution for reducing dynamic range by orders of magnitude without reducing sample throughput.
- Abundant serum protein affinity removal devices (Top6 or Top20 protein) were used to generate depleted fractions of a human serum sample in triplicate. Removal of these proteins allowed selective removal of 90 - 97% of total protein from serum.
- Tryptic digests of serum and depleted serum proteins were analyzed in triplicate by a novel LC/MS^E approach, where alternating cycles of MS and MS^E (global multiplex fragmentation) analysis provided both quantitative and qualitative information for all detectable peptide ions.
- Resulting data sets were processed by time-resolved mass alignment of the ions identified in MS and MS^E detection cycles.
- Processed data was searched against a human database, and identifications were presumed when a protein was detected by multiple peptides and a majority of replicate analyses.

SAMPLE PREP

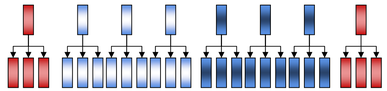
Agilent Top6 (Three Runs-One Column)



Sigma Top20 (Three Runs-Different Column Batches)



Digested Samples were Analyzed in Triplicate by LC/MS^E



INSTRUMENTATION

Samples were analyzed by LC/MS^E analysis using the Waters Protein Expression System that consisted of a nanoACQUITY UPLC™ chromatograph coupled with a Q-Tof Premier™ mass detector.

nanoACQUITY UPLC Parameters

Binary Solvent Manager (BSM)
- Solvent A: 0.1% Formic acid in MilliQ water
- Solvent B: 0.1% Formic acid in acetonitrile
- Gradient: 90 min 5 - 40 %B, 10 min 40 - 90 %B, 10 min 90 %B (wash), 30 min 5 %B (regen)
- Flow rate: 5.0 µl/min

Auxiliary Solvent Manager (ASM)
- Solvent B: 200 fmol/µl (Glu1)-Fibrinopeptide B (GFP) in 25% acetonitrile containing 0.1% formic acid
- Flow rate: 4 µl/min

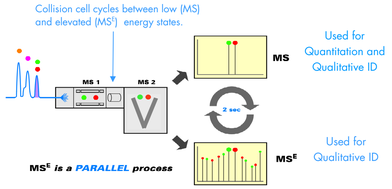
Chemistry Details

300 µ x 100 mm NanoEase Atlantis™ dC18 3µ (Waters)
Column Temperature: 35 °C

Q-Tof Premier Parameters

- Scan: ESI+ V-mode
- Resolution: >10,000
- Lockspray 1 every 30 sec
- Capillary: 3.1 kV
- Cone: 40 V
- Source: 100° C
- Collision cell:
  - MS: 4 V
  - MS^E: 15 - 40 V

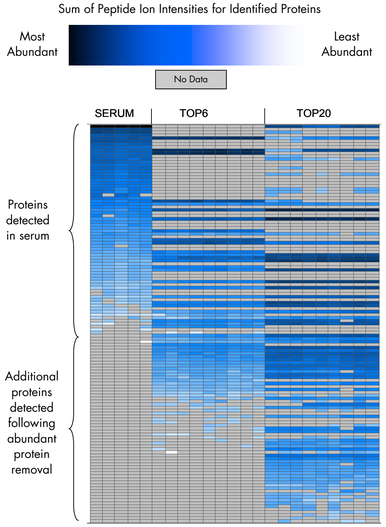
LC/MS^E ANALYSIS



LC/MS^E utilizes a cycle where there is no bias or selection of precursor ions for MS or MS^E detection (see ref. 1 and 2 for more detail). During the MS acquisition cycle, the Q- Tof collision cell is held at low potential, while in the MS^E cycle (multiplex fragmentation) the collision cell is at elevated potential. Data from MS cycles are analyzed to determine accurate peptide masses for identification and associated intensities for peptide quantitation across analyses. The MS^E spectra are complex, and represents the global fragmentation of all components detected during an MS cycle. Peptides and their fragments show identical chromatographic profiles (time-alignments), which are used to produce proper associations.

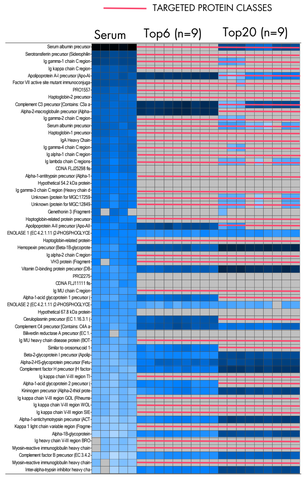
RESULTS

Effects of Top6/Top20 Protein Depletion on Identification of Serum Proteins by the Waters Protein Expression System

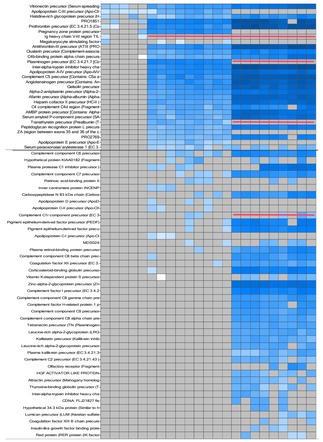


- The consistent protein profiles of nondepleted serum analyzed at the start and end of the experiment demonstrated the robustness of the LC/MS^E method over three days of data acquisition.
- Protein identifications and related intensities are observed to be reproducible across both analytical and experimental replicates in serum and depleted serum.
- Top6 protein depletion appeared highly efficient at removing a subset of proteins from the samples, and enrichment of lower-level proteins produced a significant number of new protein ID's.
- Top20 protein depletion removes a greater number of components from the sample, with slightly less efficiency, but enriches Top6 protein ID's to a much greater extent, and produces a large number of additional protein ID's from the depleted fraction.
- In some cases, Top20 targeted proteins appeared to be enriched, which arose from the presence of proteolytic processed fragments of some serum proteins (see later panels for details).

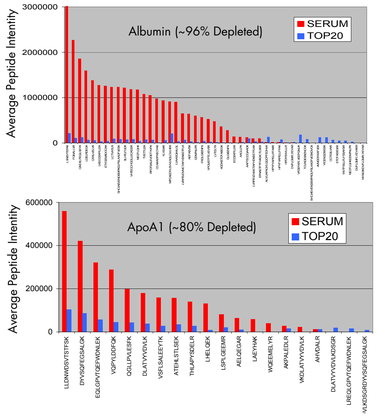
What happens to the proteins observed in Serum?



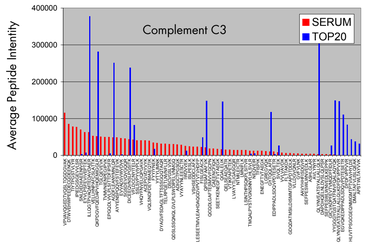
What new proteins are detected post-depletion?

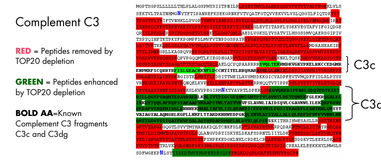


Some targeted Top20 proteins can be detected after depletion due to significant but incomplete removal.

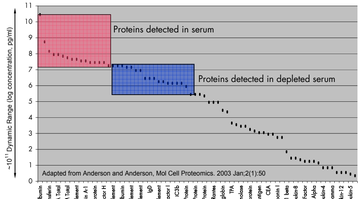


Biologically significant fragments of other Top20 depleted proteins (e.g. Complement proteins) can be detected when the parent protein is removed.





Abundant serum protein depletion (90 - 97% of total protein) predictably lowered the detection limits of serum proteins by ~2 orders of magnitude.



CONCLUSIONS

- Sample enrichment by abundant protein depletion was found to expand the effective dynamic range of LC/MS^E analysis by up to two orders of magnitude protein concentration.
- Both the Top6 and Top20 depletion workflows appear robust, and sufficient quantitative capability is retained to permit depleted serum to be used for biomarker identification.
- Samples processed using Top20 depletion produced significantly more protein identifications than the comparable Top6 depletion strategy.
- The high sequence coverage and reproducible peptide intensities obtained from LC/MS^E analysis permitted confident calculation of protein depletion efficiencies.
- In some cases, biologically relevant protein fragments could be enriched and detected following the targeted removal of the intact form(s) of the protein.
- Overall, targeted affinity depletion of high abundance serum proteins appeared to be an effective sample preparation strategy prior to LC/MS^E analysis by the Waters Protein Expression System.

REFERENCES

1. Silva, et al. Quantitative Proteomic Analysis by Accurate Mass Retention Time Pairs. *Anal Chem.* 2005, *77*, 2187 - 2200.
2. Silva, et al. Absolute Quantification of Proteins by LC/MS^E. Molecular & Cellular Proteomics. 2005, *5*, 145-156.

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