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Overview
- Sample: Human plasma
- Objectives
  - To provide a reproducible method to deplete human plasma of highly- and moderately-abundant proteins
  - To improve detection of low-abundant proteins in human plasma through one-dimensional (1D) and two-dimensional (2D) LC-MS/MS

Methods
- Depletion of the top ~60 proteins from human plasma with a ProteomeLab™ IgY-12 LC10 and SepRafil- SuperMix LC2 immunoaffinity columns
- Validation of protein concentrations by ELISA
- Denaturation, reduction, alkylation, and digestion with urea, DTT, iodoacetamide, and trypsin, respectively
- SCX fractionation with a PolysulfoethylA™ column (2.1X200 mm, 5 µm, 300 Å)

Results
- Similar Reproducibility Between Single and Tandem Depletions
- Increased dynamic range of LC-MS analyses

Enhanced Detection of Low-Abundant Proteins Utilizing Tandem Depletions

Conclusions
- Single and tandem depletions have similar reproducibility
- Tandem depletions:
  - Increase the proteome coverage by ~44–53% with LC-MS/MS analyses
  - Improve the dynamic range of LC-MS/MS analyses such that proteins in the pg/mL to ng/mL concentration range are revealed

Efficient Binding in SuperMix Column for 45 Moderately-Abundant Proteins

Table 1. Peptide identification information for two selected low abundant proteins reported to be at sub-ng/mL levels.a

<table>
<thead>
<tr>
<th>Protein</th>
<th>Identified Peptide</th>
<th>Charge State</th>
<th>Score</th>
<th>i.e.m</th>
<th>Spectral Count</th>
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<td>M-C5SFYR</td>
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References

Acknowledgments
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