**Materials and Methods**

**Sample Prep**

- **Nycodenz Sample:** Rat Liver Wild Type
- **Sucrose Sample:** Rat Liver Triton Injected
- Differential Centrifugation
- Apply ML to density gradient

**MS-method**

- **APPROACH 1:** SDS-PAGE-Tryptic digest-LCMSMS-Spectra counting
  - Digest
  - LC-MSMS
  - Spectra counting

- **APPROACH 2:** Tryptic digest-LCMSMS-Spectra counting
  - Digest
  - LC-MSMS
  - Spectra counting

- **APPROACH 3:** Tryptic digest-SCX-LCMSMS-spectra counting
  - Digest
  - SCX
  - LC-MSMS
  - Spectra counting

- **APPROACH 4:** Tryptic digest-ITRAQ labeling-SCX-LCMSMS-LC-MALDI
  - Digest
  - ITRAQ labeling
  - SCX
  - LC-MALDI
  - LC-MALDI-ITRAQ

**Equipment**

- LTQ (Thermo-Fisher) with U-3000 nano LC system (Dionex)
- 4800 MALDI TOF/TOF Analyzer with UltiMate 3000 Nano LC system

**Results**

**Comparison of MS approaches**

<table>
<thead>
<tr>
<th>Approach</th>
<th>Total Peptides</th>
<th>Unique Peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>56</td>
</tr>
<tr>
<td>2</td>
<td>115</td>
<td>120</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>56</td>
</tr>
<tr>
<td>4</td>
<td>104</td>
<td>36</td>
</tr>
</tbody>
</table>

**Examples of proteins identified in this study**

- Nycodenz Gradient
- Sucrose Gradient
- Nycodenz Sucrose
- Sucrose Nycodenz

**Conclusions**

- The lysosomal markers, as expected, showed similar relative abundances in both lysosome enriched preparations.
- Proteins that showed different abundances were considered contaminants.
- A pre-fractionation step (i.e. SDS-PAGE or SCX) enhances the quality of the data; provides more quantifiable identifications.
- All the approaches shown in this work are useful for the relative quantitative analysis of complex protein samples and show potential as a pre-classification strategy to distinguish truly lysosomal residents from contaminants.
- We consider approach #1 (SDS-PAGE-LCMSMS-Spectra counting) to be a relatively simple and straightforward method that provides very good results.

**Acknowledgements**

This work was supported by NIHDK054317