Quantitative LC/LC/MS/MS of Mixed Proteomes
Application to Studies of Human Infectious Disease and Bacterial Endosymbionts

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Label-Free Quantitation Strategy Using DIA with UPLC/UPLC/MS/MS

nanoAcquity with 2D Technology, SYNAPT HDMS

PLGS, IdentityE, Elucidator
Quantitative Proteomics to Study Metabolic and Pathogenic Properties of *Chlamydia trachomatis* Developmental Forms

*C. trachomatis* is the most common bacterial STD, and exhibits a biphasic development cycle – EB infectious, RB non-infectious
Protein Identification Metrics
(Swissprot Human, NCBI C. trachomatis, 1% FDR)

Mass spectrometry allows us to distinctly isolate the signal from Chlamydia versus Human

>54% of C. trachomatis proteome

* Mass spectrometry allows us to distinctly isolate the signal from Chlamydia versus Human
Global Strategy for Using Mass Spectrometry to Deal with Mixed Proteomes using UPLC/UPLC/MS/MS

1. **Search Against Both Human and Chlamydia DB**
   - EB: 754 Human, 3916 Chlamydia Peptides
   - RB: 4025 Human, 1274 Chlamydia Peptides

2. **Remove Peptide Matches Shared Between Species**
   - EB: 14 Homologous Peptides
   - RB: 8 Homologous Peptides

3. **Calculate fmol of Each Protein Using Method of Silva and Geromanos**
   - EB: 339 Chlamydia Proteins
     - CT842, 200 ± 25 fmol
   - RB: 181 Chlamydia Proteins
     - CT842, 47 ± 9 fmol

4. **Calculate sum ng of Chlamydia proteins, use this to normalize fmol values**
   - EB: CT842, 80 ± 3 fmol/ug
   - RB: CT842, 84 ± 19 fmol/ug
Validation of Species-Specific Quantitation using a Model System

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Sample 1 (uncorrected)</th>
<th>Sample 2 (uncorrected)</th>
<th>Sample 1 (corrected)</th>
<th>Sample 2 (corrected)</th>
<th>Measured Ratio (uncorrected)</th>
<th>Measured Ratio (corrected)</th>
<th>Theoretical Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALBU BOVIN</td>
<td>217±4</td>
<td>118±35</td>
<td>561±9</td>
<td>132±39</td>
<td>1.8</td>
<td>4.3</td>
<td>4.0</td>
</tr>
<tr>
<td>ADH1 YEAST</td>
<td>234±6</td>
<td>260±7</td>
<td>604±7</td>
<td>291±7</td>
<td>0.90</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>ENO1 YEAST</td>
<td>50±3</td>
<td>112±7</td>
<td>129±5</td>
<td>125±7</td>
<td>0.50</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>PYGM RABIT</td>
<td>27.7±0.5</td>
<td>105±5</td>
<td>69±3</td>
<td>117±5</td>
<td>0.26</td>
<td>0.60</td>
<td>0.50</td>
</tr>
<tr>
<td>E. Coli Proteins (average)</td>
<td>109±6</td>
<td>263±17</td>
<td>281±12</td>
<td>294±19</td>
<td>0.41</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Reproducibility of Protein Quantitation

Protein CV Distribution, EB

Protein CV Distribution, RB
Species-Specific Correction Applied to *Chlamydia* Protein Quantitation

EB vs RB Quantitation
(with Species-Specific Scaling)

Select Proteins with Verification

<table>
<thead>
<tr>
<th>Immunoblot</th>
<th>Mass spectrometry$^1$</th>
<th>Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EB</td>
<td>RB</td>
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<tr>
<td>RpoD</td>
<td>69.6 ± 1.2</td>
<td>64.9 ± 8.6</td>
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<tr>
<td>RpoB</td>
<td>128.1 ± 4.2</td>
<td>200.1 ± 38.0</td>
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<tr>
<td>PmpD</td>
<td>10.8 ± 1.2</td>
<td>368.6 ± 45.4</td>
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<tr>
<td>IncG</td>
<td>ND</td>
<td>67.6 ± 14.8</td>
</tr>
<tr>
<td>CT288</td>
<td>67.2 ± 2.0</td>
<td>ND</td>
</tr>
<tr>
<td>Hc1</td>
<td>29.7 ± 5.1</td>
<td>ND</td>
</tr>
</tbody>
</table>
- EBs are enriched in **T3S-effectors and chaperones**, as well as in enzymes involved in **glucose catabolism**.

- RBs are enriched for **protein synthesis and assembly** components, **ATP generation** and transport, and **nutrient import**.
Molecular Evidence of the Different Metabolic Properties of the two Developmental Stages

Proteomic results show the EB and RB proteomes are streamlined for their function - maximum infectivity for EB, replicative capacity for RBs.
Proteomic Analysis of an Unculturable Bacterial Endosymbiont *Blochmannia* Bacteria in *Camponotini* Ants

- Many groups of unculturable bacteria perform key ecological functions within the context of close associations with animal hosts.
- Insects are particularly prone to establishing long-term relationships with intracellular bacterial symbionts that perform key nutritional functions.
- Here studied were *Blochmannia* bacteria in the ant tribe *Camponotini*.
  - The bacteria provide nutritional support and thus allow hosts to survive in ecological niches otherwise inaccessible to them.
  - Conversely, the insect hosts are thought to provide persistent, stable and nutrient-rich habitats to the endosymbionts.

- These intracellular microbial associates have become models to study the evolution and functioning of intimate bacterial-animal mutualisms.
Proteomic Analysis of an Unculturable Bacterial Endosymbiont Blochmannia Bacteria in Camponotini Ants

- Midgut from carpenter ants dissected and pooled (N=6)
- Homogenized pool filtered (20 um) to remove cells larger than Blochmannia
- Filtrate pelleted, washed and pellet lysed using RapiGest (Waters).
- Protein concentration normalized, then reduced, alkylated and digested
- UPLC/UPLC/MS/MS quantitation accomplished using ion-mobility assisted DIA (HD-MS²)
  - 2D nanoAcquity
  - SYNAPT G2 HDMS
- ‘Top 3’ quantitation method used to calculate fmol/ug quantities of proteins

*Blochmannia* in an ant cell
Use of UPLC/UPLC with HD-DIA for the Analysis of Unculturable Bacterial Endosymbiont

The DDA, MSE, and HDMS\textsuperscript{E} data from three \textit{B. chromaiodes} Peptide FDR was 1.2% for both ant host and bacterial peptides.
Comparison of Genome, Proteome Content, and Protein Abundances according to Functional Categories
Conclusions

- UPLC/UPLC/MS/MS provides good overall proteome coverage
  > 54% of predicted proteins in *C. trachomatis* and *Blochmannia* proteomes
- Data-Independent Analyses with ‘Top 3’ quantitation proved reproducible and allows ‘Species Specific’ quantitation from time-dependent studies of infectious disease samples
- These quantitative studies show proteomics to provide unique insights into ‘mixed proteome’ samples from infectious disease and intimate symbiosis.
  – translation from genomics to functional proteomics
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