1 Motivation

- **Metaproteomics** uses mass spectrometry to gain taxonomic and functional information on a microbiome.
- Traditionally, **Data-Dependent Analysis (DDA)** mass spectrometry has been used to sequence peptides in bottom-up proteomics.
- DDA is a stochastic process which is dependent on the most abundant ions entering into the mass analyzer, resulting in a lack of reproducibility for low-abundance proteins.

2 Hypothesis

- Data-Independent Acquisition (DIA) mass spectrometry fragments all ions entering the mass analyzer.
- DIA-MS provides more reproducible measurements of low abundance proteins than DDA-MS resulting in deeper sequencing.
- **DIA-MS results in more protein identifications than DDA-MS for metaproteomic applications.**

3 Samples & Methods

- **Samples:** 1µg replicates of constructed microbial communities (see below)
- **LC:** NanoLC column packed with C18 resin run on Ultimate 3000 UHPLC with a 90-minute gradient
- **MS:** QExactive Orbitrap Quadrupole Hybrid Mass Spectrometer; Orbitrap Fusion Tribrid Mass Spectrometer; Orbitrap Eclipse with FAIMS Pro
- **Proteome Discoverer 2.5:** SEQUEST HT, Percolator nodes for DDA-MS data analysis
- **EncyclopeDIA, DIA-NN,** and **Spectronaut** software suites were utilized for DIA-MS data analysis

4 Artificial Microbiome Design

![Experimental Design. Three microbial communities of differing composition are analyzed via three separate Orbitrap LC-MS systems.](image)

5 More Proteins Detected in DIA-MS

DIA-MS runs generally result in higher amounts of proteins detected. In addition, DIA-MS runs show significantly higher degrees of overlap between replicates.

6 DIA-MS Allows for Deeper Sequencing

In all laboratories, the use of DIA-MS allows for the detection of low-abundance phage proteins invisible to DDA-MS analyses.

7 Quantitative Accuracy of DIA, DDA

![Comparison of observed and known protein abundances for DIA and DDA analyses.](image)

8 Discussion / Recommendations

- Data-Independent Acquisition (DIA) was examined in multiple instruments to determine if it would provide improved sequencing over Data-Dependent Acquisition (DDA).
- DIA-MS resulted in the detection of more proteins more reproducibly than DDA-MS.
- DIA-MS allows for the detection of low-abundance phage proteins.
- DIA-MS results in the more accurate quantitation in low-resolution mass spectrometers.
- Future experiments will use DIA-MS to characterize human and environmental microbiota in response to stimuli.

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