

Non-ribosomal protein quantitation in sucrose gradient fractions

Sample preparation

Experimental sample
Sucrose gradient fraction from ^{14}N -metabolically labeled cell lysate

Tryptic digest
Mixed, reduced, alkylated, digested, PepClean purified

Reference sample
 ^{15}N -metabolically labeled cell lysate

MS data acquisition

Data acquisition [Analyst]
SWATH-MS² data-independent acquisition [see attached]

Raw data files
.wiff files [vendor format]

Ion chromatogram extraction

Proteins of interest
List of proteins of interest [selected list of factors – see Fig S3]

Chromatographic peak extraction [Skyline]
MS² MRM-like transitions extracted for each product ion of interest. Extractions limited to 5 minute window around expected retention time based on iRT calibration. 8 most intense product ions in spectral library extracted per precursor ion.

Spectral library
Consensus spectral library [generated by SpectraST, see WF2]

Extracted ion chromatograms
Ion intensity vs. retention time
.skyd file

Peak filtering

Spectral interference filtering [Skyline]
Each transition hand-inspected for signal intensity, spectral and chromatographic interference

Automated filtering [Python scripts]
Extracted ion chromatograms filtered for exact mass error, deviation in expected retention time, spectral dot product between ^{14}N and ^{15}N , spectral dot product with library spectra

Quantitation

Transition intensity list [.csv]
 ^{14}N and ^{15}N extracted ion chromatographic intensity for each product ion, for each peptide, for each protein.

Peptide relative abundance [Python]
All ^{14}N product ion intensities summed. All ^{15}N product ion intensities summed. Ratio of $^{14}\text{N}/^{15}\text{N}$ intensity determined.

Protein relative abundance [Python]
For each protein, the median abundance ratio across all children peptides reported, normalized to largest ratio observed [DeaD].

Relative protein abundance [.csv]
A relative protein abundance .csv file. Plotted as a heatmap in Fig S3C

Experimental technique

Software tool

Data file