Description of time course investigation of central carbon metabolism of *S. cerevisiae*

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1 Experimental design

The investigation is discussed in details in (Picottii et. al., 2009). It aimed at studying the central carbon metabolism of *S. cerevisiae*, and was conducted in a time course experimental design. Three biological replicates were analyzed at ten time points (T1-T10), while yeasts transited through exponential growth in a glucose-rich medium (T1-T4), diauxic shift (T5-T6), post-diauxic phase (T7-T9), and stationary phase (T10). The experiment targeted 45 proteins in the glycolysis/gluconeogenesis/TCA cycle/glyoxylate cycle network, which spans the range of protein abundance from less than 128 to 10E6 copies/cell.

2 Sample selection

The samples were mixed prior to trypsinization with an equal amount of proteins from ^{15}N-labeled yeast cells, and the labeled proteins were used as a reference before the MS analysis.

3 Sample preparation

Two venous blood samples (12ml) were collected per patient in EDTA blood tubes (BD Vacutainer, 0.184M EDTA, BD Diagnostics, Franklin Lakes, US) and stored on ice for a maximum of three hours until further processing. Blood samples were centrifuged at 3000g for ten minutes at 4°C, and aliquots of the supernatant plasma frozen at -80°C. Specimen were subjected to N-glycoprotein enrichment[1] and trypsin digestion. Heavy-labeld AQUA[2] peptides were spiked into each plasma sample as an internal reference before the MS analysis.

4 Mass spectrometric analysis

Each sample was measured in a single mass spectrometry run with no technical replicates, resulting in a total of 30 mass spectrometry runs. Each protein was represented by 1-2 peptides, and each peptide was represented by 2-3 transitions[3].

5 Signal processing

The original study by (Picotti et. al., 2009) manually quantified each transition. Since this manuscript aims at an automated analysis workflow, here we quantified each transition by its peak apex automatically using MultiQuant software package (Applied Biosystems/MDS Sciex), without additional manual curation. The procedure resulted in the total of 236 quantified transitions with no missing values. These quantifications likely contain more variation than the manually quantified transitions in the original investigation.

1. Zhang, H., Li, X., Martin, D. & Aebersold, R. Identification and quantification of N-linked glycoproteins using hydrazide chemistry, stable isotope labeling and mass spectrometry. *Nature Biotechnology* **21**, 600–666 (2003).

2. Gerber, S. A., Rush, J., Stemman, O., Kirschner, M. W. & Gygi, S. P. Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. *Proceedings of the National Academy of Science of the USA* **100**, 6940–6945 (2003).

3. Picotti, P. *et al.* High-throughput generation of selected reaction-monitoring assays for proteins and proteomes. *Nature Methods* **10**, 43–46 (2010).