SHIMADZU
Excellence in Science

Application Data Sheet

No.50

LC-MS
Liquid Chromatograph Mass Spectrometer

Quantitative Proteomics of Metabolic Enzymes in *S. cerevisiae* Using Triple Quadrupole LC/MS/MS

[LCMS-8040]

Using microorganisms such as S.cerevisiae to produce materials has been applied across many industries such as fermented foods, chemicals, pharmaceuticals, and biofuels. These microbial cell factories are metabolically orchestrated by regulating expression levels of enzymatic proteins. To understand these metabolic pathways, it is important to monitor enzyme abundances. An analytical technique used for targeted proteomics utilizes LC-MS/MS to monitor pre-selected tryptic peptides generated from these proteins of interest. Multiple reaction monitoring (MRM) of these peptide transitions provides high sensitivity and specificity and is a powerful tool for quantification of proteins in biological samples. This LC-MS based approach does not require antibodies for each protein or peptide analyzed as ELISA does. On the other hand, to be effective, the mass spectrometer must be capable of high-speed scanning to monitor the many tryptic peptides derived from a complex mixture of proteins. The high scan speed capability of Shimadzu UFMS technologies can reveal the dynamic behavior of many proteins from these biological samples[1].

Wild type strain S288C and single gene deletion mutants of strain BY4742 were cultured in media containing either non-labeled or labeled glucose for comparative proteomics analysis. Here, 303 peptides from 137 proteins from strain S288C and 409 peptides derived from 199 proteins from strains of BY4742 were monitored, totaling 2856 MRM transitions from both strains. Figure 1 shows MRM chromatograms of tryptic peptides (SIIGATSIEDFISK) derived from Gnd1P extracted from these wild type and single gene deletion mutants. Y-series ions observed in the labeled and non-labeled peptides provide the basis for comparison (Figure 1 A, B). The expression level of Gnd1P was changed in three mutants relative to the wild type, especially BY4742gnd1Δ, which, as expected, was greatly reduced (Figure 1 C-E).

In Figure 2, relative enzyme levels of wild type and gene mutant strains measured at different dwell times are compared. Reproducible data can be acquired without compromise even at high-speed conditions using a 1 msec pause and 1 msec dwell time, thus confirming the benefit of UFMS technologies.

The expression levels of Pfk1p, Zwf1p, and Gnd1p were further reduced in the gene deleted strains, thereby reflecting each mutated genotype, whereas the growth curve of strain pfk1Δ was similar to the wild type. Conversely, several enzyme expression levels were relatively increased. It has been predicted that the zwf1Δ strain behaves similarly to the gnd1Δ strain because Zwf1 and Gnd1 both participate in NADPH regeneration; however, in this study, the profiles for these two proteins differed.

These results show the strength of UFMS technologies for quantitative analysis tracing targeted protein expression levels from biological samples.

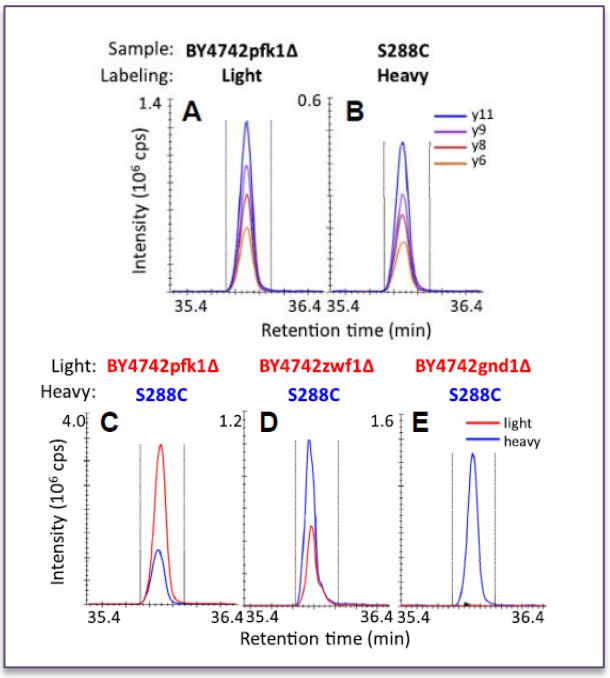

Figure 1: Chromatograms of Gnd1p in BY4742pfk1Δ strain cultured with non-labeled glucose (A), wild type cultured with labeled glucose (B), differing expression levels (C-E)

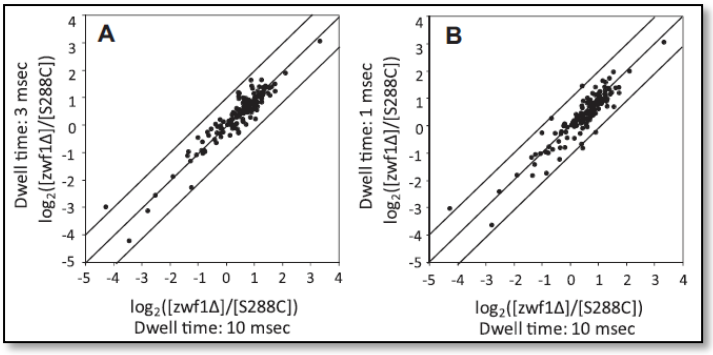

Figure 2: Effect of dwell times on peptide quantification.

Table 1: Analytical conditions

HPLC system:	Prominence Nano
MS system:	LCMS-8040 with Nano ESI interface*
Mobile phase A:	0.1% formic acid in water
mobile phase B:	0.1% formic acid in acetonitrile
Flow rate:	400 nL/min
Gradient:	10%B(0-10 min)>40%B(45 min)
Analytical column:	L-Column ODS (0.1 mmI.D.x150 mmL.)
S/MRM condition:	MRM library for metabolic enzymes in yeast.

*Please contact us for details about Nano ESI.

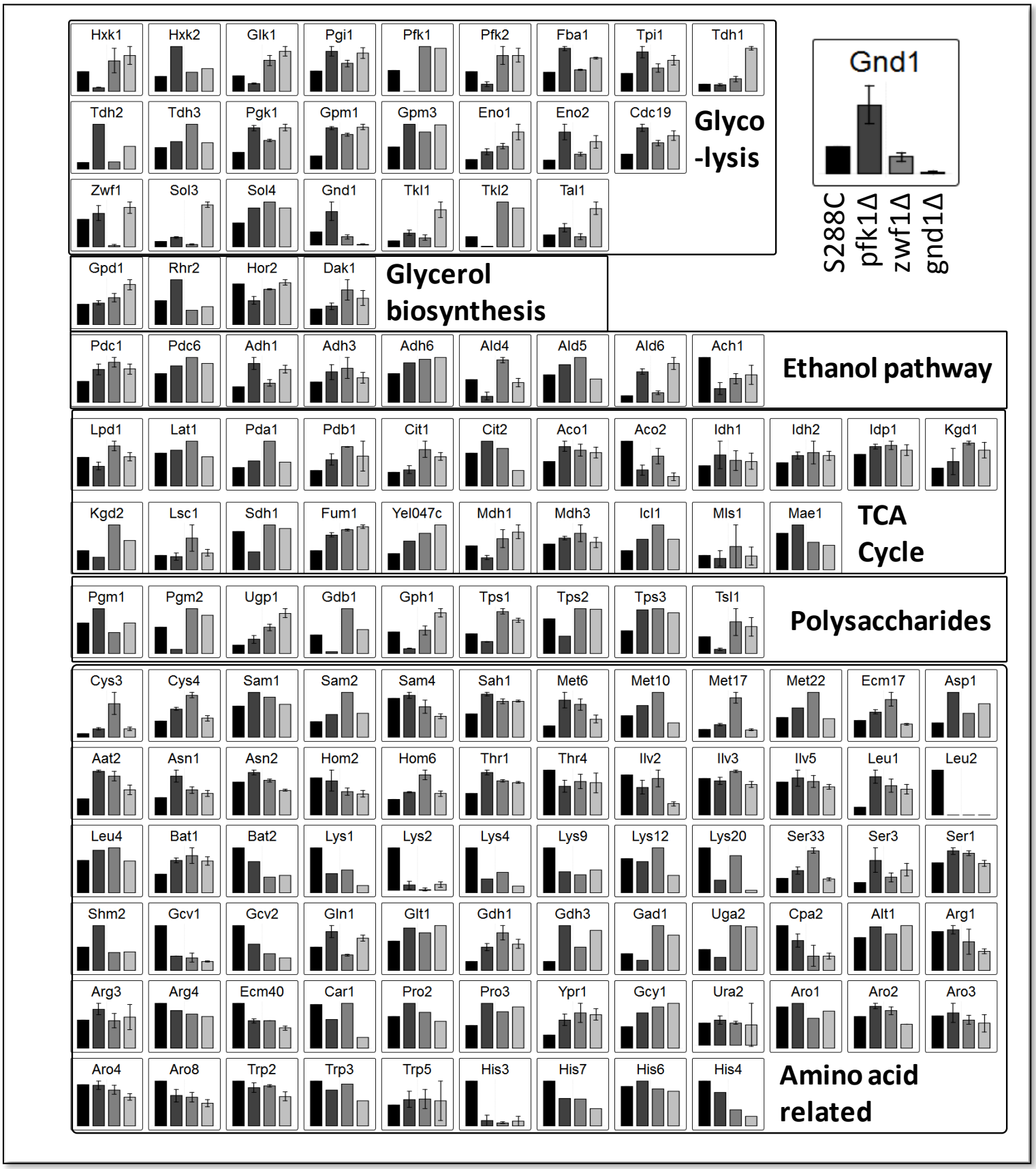

Figure 3: Profile of 137 metabolic enzymes from the wild type compared to three single gene deletion mutants.

Reference) Matsuda F, Ogura T, Tomita A, Hirano I, Shimizu H. Nano-scale liquid chromatography coupled to tandem mass spectrometry using the multiple reaction monitoring mode based quantitative platform for analyzing multiple enzymes associated with central metabolic pathways of *Saccharomyces cerevisiae* using ultra fast mass spectrometry. *J Biosci Bioeng.* 2015 Jan;**119** (1) :117-20.

First Edition: February, 2015

Shimadzu Corporation
www.shimadzu.com/an/