Time-resolved analysis of proteome dynamics by tandem mass tags and stable isotope labeling in cell culture (TMT-SILAC) hyperplexing

Welle KA, Zhang T, Hryhorenko JR, Shen S, Qu J, Ghaemmaghami S (2016) *Mol Cell Proteomics* 15:3551–3563.

The protein composition of a cell at any given time defines the cell's health and function. A specific protein's abundance, localization, and lifetime has traditionally been studied with antibodies or other highly selective tags. In order to conduct a global analysis of protein dynamics, Welle and coworkers recently described a method that employs metabolic isotopic labeling (stable isotope labeling in cell culture or SILAC) in combination with isobaric tagging for the multiplexed mass spectrometry analysis of complex protein samples at different time points.

In recent years, the field of mass spectrometry has exploded with advances in both instrumentation and methodology that allow quantitative analysis of complex mixtures. Tandem mass spectrometry (LC-MS/MS) provides a means of defining the composition, expression levels, and modifications of proteins in a given sample at a precise time point. This proteome snapshot, however, cannot capture the dynamics of protein expression, including the kinetics of protein synthesis and clearance and the ongoing modifications of proteins that define their function.

Welle and coworkers have addressed the limitations inherent in a static proteome analysis by combining dynamic SILAC experiments with isobaric tagging to produce highly multiplexed (i.e., hyperplexed) samples that can be analyzed in a single mass spectrometry run. SILAC is a standard protocol for metabolically incorporating $^{15}N/^{13}C$-labeled amino acids into proteins and then analyzing these isotope labels using LC-MS/MS. Dynamic SILAC further integrates a time component into the labeling—either by pulsing the labeled amino acids in cell culture media or by sampling the cells over time—to allow proteome-wide determination of protein synthesis and degradation kinetics. In the method reported here, samples acquired at each time point were labeled with a unique isobaric tag (tandem mass tag or TMT) before combining them into a single hyperplexed sample for analysis by LC-MS/MS.

To determine the effectiveness of this method, these researchers used the combination of SILAC and TMT labeling with LC-MS/MS analysis to measure the kinetics of protein turnover for over 3,000 proteins in human dermal fibroblasts and compared the results with those obtained using non-multiplexed methods. They obtained similar rates for protein synthesis and degradation when time points were analyzed with either TMT-SILAC hyperplexing within a single mass spectrometry run or dynamic SILAC across multiple mass spectrometry runs.

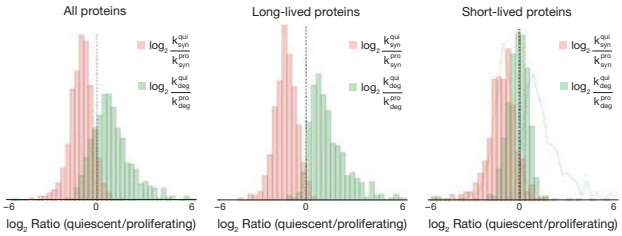

Figure 1. The use of TMT-SILAC hyperplexing to measure relative differences in synthesis rates (k_{syn}) and degradation rates (k_{deg}) between quiescent and proliferating cells. Histograms of the distribution of log_2 ratios of k_{syn} and k_{deg} between quiescent and dividing cells are shown for all proteins, long-lived proteins (half-lives >3 days in proliferating cells) and short-lived proteins (half-lives <3 days in proliferating cells). The dotted lines on the rightmost plot indicate the distribution of long-lived proteins for comparison. Reprinted with permission from Welle KA, Zhang T, Hryhorenko JR et al. (2016) *Mol Cell Proteomics* 15:3551–3563.

Dynamic SILAC provided slightly better precision, but the TMT-SILAC hyperplexing method was improved by adding synchronous precursor selection (SPS) to minimize interference from co-isolated interfering ions.

Welle and coworkers then combined their TMT-SILAC samples acquired at different time points from both quiescent and proliferating fibroblasts into a single hyperplexed sample to simultaneously measure the ratios of synthesis and degradation rate constants in these two cell states (Figure 1). As expected, they found that protein synthesis was globally decreased in quiescent cells compared with that in proliferating cells, presumably to compensate for the lack of cellular growth. They also observed that protein degradation rates were increased in quiescent cells, particularly for long-lived proteins. They note that this study is the first quantitative global census of protein synthesis and protein degradation rates in quiescent cells. Their experiments demonstrate that the use of TMT-SILAC hyperplexing with a single LC-MS/MS run should simplify the time-resolved analysis of proteome dynamics in different cell environments or in reaction to different stimuli, while also reducing the time and costs associated with multiple mass spectrometry runs. ■

Product	Quantity	Cat. No.
MEM for SILAC	500 mL	88368
Pierce™ C18 Spin Tips	96 tips	84850
Pierce™ High pH Reversed-Phase Peptide Fractionation Kit	12 reactions	84868
Pierce™ Trypsin Protease, MS Grade	5 x 20 µg	90057
TMT10plex™ Isobaric Label Reagent Set	30 reactions	90111