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APPLICATION NOTE 38

Stable Isotope Dimethyl Labeling

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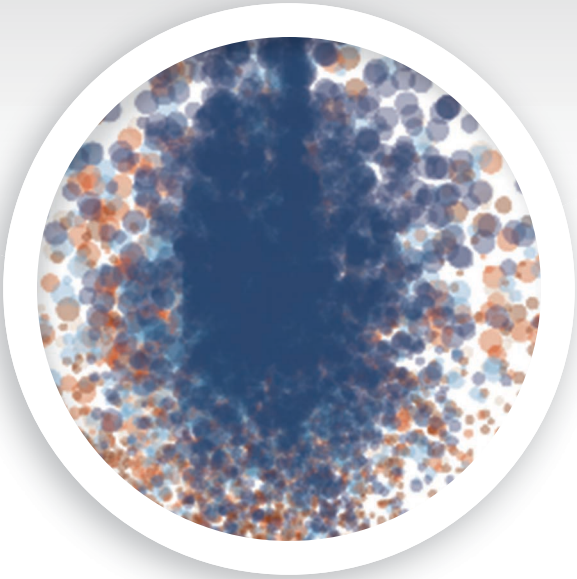

"Unlike SILAC and SILAM, dimethyl labeling is an easy-to-manage labeling technique that can be applied cost-effectively to almost any type of sample, including samples such as body fluids and tissue."

Albert J.R. Heck, Prof. Dr.
Department of Biomolecular Mass Spectrometry and Proteomics
University of Utrecht

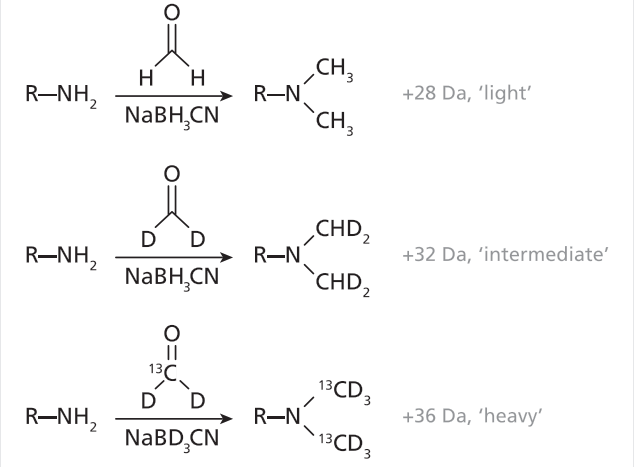

Figure 1.

The quantitative analysis of proteomes is an increasingly important aspect of mass spectrometry (MS)-based proteomics. The most commonly used methods for comparing and accurately quantifying protein levels rely on the use of differential isotopic labeling. Proteins or peptides from different samples are labeled using compounds with near identical chemical properties yet each containing a unique stable isotope composition resulting in different masses. This way, the different samples can be combined and still be distinguished in a single MS analysis. The stable isotopes can be introduced by chemical labeling at the protein or peptide level with isotopomeric tags. This method is particularly suited for tissue samples derived from animals or humans where metabolic incorporation is difficult.

Stable isotope dimethyl labeling is a very straightforward, fast and inexpensive quantitative proteomics method.[1] In this strategy, all primary amines (the *N*-terminus and the side chain of lysine residues) in a peptide mixture are converted to dimethylamines (Figure 1). By using combinations of several isotopomers of formaldehyde and cyanoborohydride, peptide triplets can be obtained that differ in mass by a minimum of 4 Da between the different samples. A number of facile protocols have been developed to allow its use in most proteomic applications.[2] The suitability of stable isotope dimethyl labeling for quantitative mass spectrometry has been demonstrated in several studies and has been shown to be quite competitive when compared to other popular strategies.[3] It has been used to compare proteomes,[4] phosphoproteomes[5] and the results of affinity purifications[6] to name but a few. As an example, Munoz, et al.,[4] utilized dimethyl labeling to ascertain how similar are embryonic stem cells to the proteomes of induced pluripotent stem (iPS) cells and their precursor fibroblasts cell lines (Figure 2). In their case, they were comparing the proteomes of two fibroblast cell lines and two induced pluripotent stem cell lines to an embryonic stem cell line. Most stem cell types require a nonstandard culturing condition which means that a classical technique such as SILAC is difficult to implement due to the need of fundamentally changing the media used for the culturing conditions. On the other hand, dimethyl labeling is an efficient strategy that is applied once the sample is

(continued)

digested and so demands little change in the preparation of a cellular system. Additionally, the dimethyl reagents themselves are compatible with all common digestion protocols and, essentially, invisible to downstream sample preparation steps.

In this example, Munoz, et al., performed a cell lysate-level digest prior to labeling and then performed SCX fractionation followed by LCMS using both electron transfer dissociation (ETD) and collision induced dissociation (CID) for peptide sequencing. Dimethyl labeling has no issues with SCX and is, in fact, beneficial for ETD and CID where spectra are often better. The experiment was performed as a triplicate labeling experiment. One label was used for the fibroblast cell line, one for the iPS cells and the third for embryonic stem cells. The end result was four samples; two sets of cell lines and two replicates. The data was processed using Proteome Discoverer (Thermo), but MaxQuant is an excellent, freely available, alternative software solution. The resulting data corresponded to one of the largest sets of proteomes identified with over 10,000 protein groups identified and quantification of >6000 proteins per experiment (Figure 2). The expression levels of the vast majority of proteins were very similar between induced pluripotent stem cells and embryonic cells. The proteome profiles suggested the two types of stem cells were very similar BUT not the same and thus further confirming studies that characterized, for example, mRNA levels, microRNA levels and CpG islands.

Dimethyl labeling is a cost-effective way of performing quantitative proteomics and is at home with generating pilot study data to state-of-the-art characterizations.

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CDLM-4599	Formaldehyde (^{13}C, 99%; D_2, 98%) 20% w/w in D_2O
DLM-7364	Sodium cyanoborodeuteride D_3, 98%
ULM-9497	Sodium cyanoborohydride (unlabeled) CP 95%
ULM-9498	Formaldehyde (unlabeled) 20% w/w in H_2O – contains 10-15% methanol

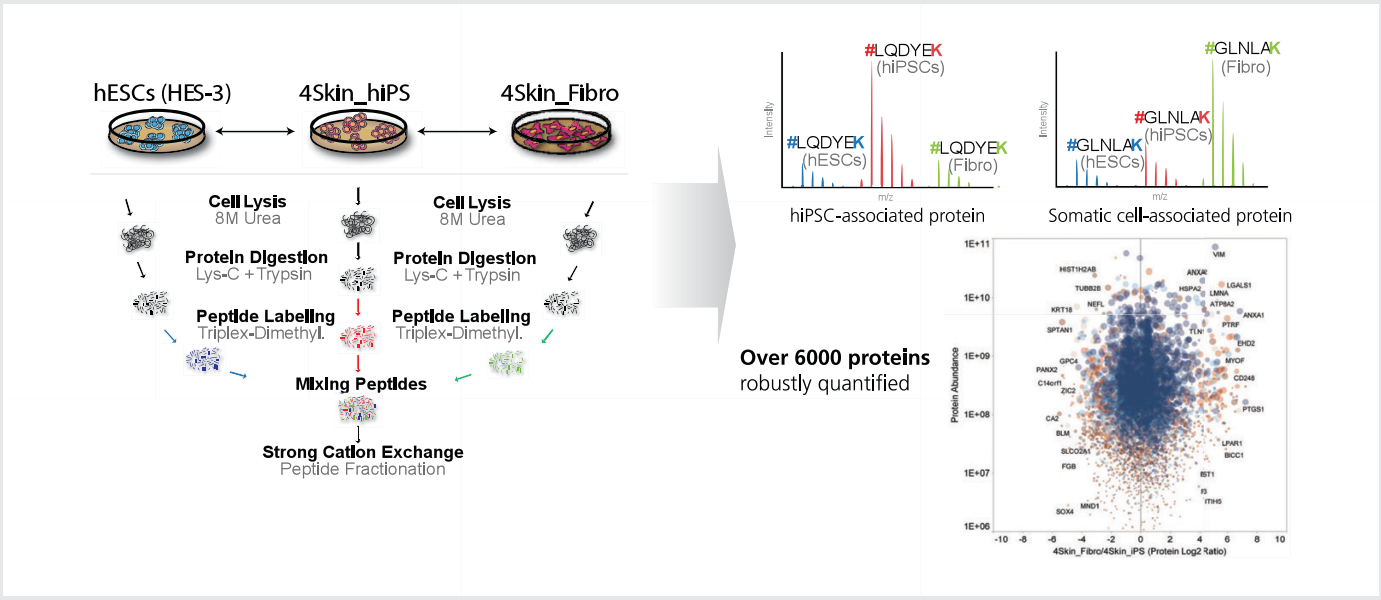

Figure 2. (Left) Illustrative experimental design for a quantitative proteomics experiment based on stable isotope dimethyl labeling, as used for a quantitative proteome profiling of hiPSCs and hESCs. (Right, top) The peak intensities of the identified peptides are proportional to their abundance. (Right, bottom) Global quantitative proteomic comparison of hiPSCs and its precursor fibroblast cell line. The absolute protein abundance (log10 scale) is plotted against the relative protein ratios (log2 scale). All data adapted from Munoz, et al.[4]

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