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A SYSTEMATIC APPROACH TO EVALUATE METABOLIC CONVERSIONS OF AMINO ACIDS IN SILAC EXPERIMENTS

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SILAC is an established approach for quantitative proteomics where proteins are labeled with stable isotopes during cell culture. A major drawback of this technique is the metabolic conversion of labeled amino acids that may hamper accurate quantification. An illustrative example of this phenomenon is the generation of labeled proline from arginine, known to occur in a good number of biological models. We propose a novel methodology to characterize and quantitate such conversion events as well as to evaluate labeling efficiency in SILAC experiments. Our strategy makes use of acid hydrolysis to reduce a complex sample of proteins to a much simpler mixture of amino acids that is then analyzed by LC-MS. Since it is carried out at the amino acid level, tracking the fate of the isotope label is straightforward and can be performed for each amino acid independently. After employing this method with several mammalian cell lines, grown in the presence of heavy lysine or arginine, labeling efficiency and amino acid conversions could be accurately assessed. Only arginine to proline conversion was found to occur at a significant extent, with large differences among the cell lines tested. Finally, increasing proline concentration in the growing medium was shown to prevent arginine conversion without any marked side effect.