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Perspective

Tandem Mass Tags (TMT) as an Important Isobaric Labelling Method for Protein Analysis

Alexander Dayon*

Department of Medicine, University of Geneva, Geneva, Switzerland

INTRODUCTION

Tandem Mass Tags (TMT) are chemical tags that facilitate sample multiplexing for Mass Spectrometry (MS) based quantification and identification of biopolymers such as proteins, peptides, and nucleic acids. TMT belongs to a family of reagents called isobaric mass tags. This is a set of molecules with the same mass, but producing reporter ions with different masses after fragmentation. The relative proportions of measured reporter ions represent the relative abundance of the labelled molecule, but ion suppression adversely affects accuracy. MALDI-TOF-based protein fingerprinting is used to digest the sample with a specific protease (usually trypsin) and acquire an MS spectrum that produces the masses of all peptides (or MH^+). These qualities are used as fingerprints for searching proteins in the database and matching them with their measured masses. Protein tags are convenient and useful tools to improve recombinant protein solubility, streamline protein purification, and easily track proteins during protein expression and purification. Isobaric labeling (such as TMT and iTRAQ) has become a versatile technique for relative protein quantification by testing up to 10 biological samples simultaneously in a single run. This makes high-throughput protein quantification fast and easy. For accurate quantitative results, TMT labeling efficiency should be examined to ensure that all samples are fully labeled with TMT. This is important because inadequate amounts of TMT label used, changes in pH, or protein quantification can lead to inefficient labeling and adversely affect subsequent results. It's a step iTRAQ uses isobaric tags, originally called tandem mass tags, that have identical mass and chemical properties. Therefore, heavy and light isotopes co-elute during LC separation. These tags are removed from the peptides during the LC-MS/MS run of CID. A distinct advantage of these bulk isobaric labels is the flexibility provided in label selection and multiplexing capabilities. Despite these challenges, TMT based proteomics has been shown to offer greater precision than label-free quantification. The TMT tag not only aids in protein quantification, but can also enhance the detection sensitivity of certain highly hydrophilic analytes such as phosphopeptides in RPLC-MS analysis. The tag contains four regions: Mass reporter region (M), cleavable linker region (F), mass normalization region (N), and protein reactive group (R).

DESCRIPTION

Although all tags have identical chemical structures, each tag contains isotopes substituted at different positions, so the mass reporter and mass normalization region have different molecular weights in each tag. Since the combined M-F-N-R regions of the tags have the same overall molecular mass and structure, molecules labeled with different tags are indistinguishable during chromatographic or electrophoretic separation and in single MS mode. Fragmentation in MS/MS mode obtains sequence information from fragmentation of the peptide backbone and quantification data from fragmentation of the tag simultaneously to generate mass reporter ions. The structure of the TMT tag has been published through the unimod database at unimod.org, allowing mass spectrometry software such as Mascot to describe the mass of the tag. TMT tags are commonly used to label samples of equal abundance and sizes of proteins. Tandem Mass Spectrometry (MS/MS) involves two separate stages of mass analysis and can be used to decipher relationships among ions in a mass spectrum or to identify compounds in complex mixtures that have not been subjected to prior separation. Isobaric labeling *via* Tandem Mass Tag (TMT) reagents helps the researchers in sample multiplexing prior to LC–MS/MS, facilitating high-throughput large-scale quantitative and qualitative proteomics. Consistent and efficient labelling reactions are essential to achieve robust quantification; therefore, embedded in our clinical proteomic protocol is a Quality Control (QC) sample that contains a small aliquot from each sample within a TMT set, known as “mixing QC.” This mixing QC enables the detection of TMT labeling issues by LC–MS/MS before combining the full samples to allow for salvaging of poor TMT labelling reactions. Relabeling does not always rescue the TMT reaction and peptide samples may remain acidic after suspension in 50 mm HEPES buffer (pH 8.5), resulting in low Labeling Efficiency (LE). To obtain a more robust TMT labeling method, it is researched that the absence of reporter ions, the ratio of

Correspondence to: Alexander Dayon, Department of Medicine, University of Geneva, Geneva, Switzerland; E-mail: alexander.dayon@unige.ch

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average TMT set MRII to individual channel MRII, and the log2 reporter ion ratio from mixed QC samples. It was also found out that sample pH was a key factor in LE, and increasing the buffer concentration of poorly labelled samples prior to relabeling successfully rescued the TMT labeling reaction.

CONCLUSION

Furthermore, resuspension of peptides in 500 mm HEPES buffer for TMT labeling resulted in consistently higher LEs and lower missing data. By better controlling sample pH for labeling and implementing multiple methods to assess labeling quality prior to sample binding, robust TMT labeling is achievable in large-scale quantitative studies. In recent years, the development and refinement of isobaric labeling strategies (chemical derivatization of proteins and peptides using isobaric chemical tags) has allowed scientist to overcome some of its limitations. Efficient, robust, and consistent TMT labeling reactions are critical for accurate quantitative proteomics measurements. TMT is highly pH dependent. Therefore, using a protocol with high concentrations of HEPES buffer (pH 8.5) (e.g., 200 mm-500 mm) is a simple and inexpensive way to prevent poor labeling reactions. Although not tested here, we believe these results are relevant to other NHS-based labeling reagents (such as iTRAQ) in terms of the effect of buffer pH on labeling quality.