

## **TMT CALIBRATOR: THE UTILITY OF TANDEM MASS TAGS TO GENERATE QUANTITATIVE MASS SPECTROMETRIC ASSAYS WITH MULTI-POINT CALIBRATION CURVES**

<sup>1</sup>WARD M., <sup>1</sup>PIKE I., <sup>2</sup>KUHN K., <sup>2</sup>KIENLE S., <sup>2</sup>BAUMANN C., <sup>1</sup>SCHOFIELD E.,  
<sup>1</sup>BYERS H., <sup>2</sup>SCHWARZ J., <sup>1,2</sup>SCHULZ-KNAPPE P.

<sup>1</sup>*Proteome Sciences plc, Cobham, UK.* <sup>2</sup>*Proteome Sciences, Frankfurt am Main, Germany.*

As it seems common for novel technologies, proteomics now starts to mature by adapting rules and regulations of established technologies. As a key technology for peptide and protein biomarker discovery and development, the limitations of restricted reproducibility and rather poor quantitative performance are starting to be cured by specific “bits and pieces”.

Here we attempt to add a further improvement to the measurement of peptides and proteins from complex biological mixtures such as cellular extracts and body fluids.

One of the new and most promising strategies for biomarker discovery and development is to use mass spectrometry combined with quantitative tags for analysis. The idea behind the use of such tags is that the quantitative ratio between individual proteins remains constant after mixing of several tagged samples. The conservation of relative proportions is a key for subsequent separation and quantitative analysis.

Proteome Sciences has pioneered the field of isobaric mass tags. We use Tandem Mass Tags (TMT<sup>®</sup>) and currently label amine functions in proteins and peptides on N-termini and Lysine amino acids. The tags have the same overall mass and identical physico-chemical properties. Labeled proteins behave identically during all pre-Mass-Spec steps such as freeze-thawing, sample handling and chromatography. The mass tags release individual reporters during MS/MS which indicate the proteins' origin. By labeling a reference peptide with several different tags, the user is able to generate custom-made calibration curves to allow for 1-to 5-point calibration of analytes over diverse dynamic ranges with excellent assay characteristics matching those of individual ELISA-type assays. Examples of the general principle are given as well as specific applications in tissues and body fluids.