A new approach for defining the relative contribution of co-migrating proteins to spot intensity in 2D-PAGE

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Introduction

When using 2D PAGE, co-migrating proteins can present a major problem for quantitative protein expression comparison. It has been described that up to six proteins may appear in a single spot [1]. When two or more proteins are identified in a spot differentially displayed between two sample groups, it becomes very time consuming to discover which one is up- or down regulated. To tackle this problem we tested two different labeling techniques, iTRAQ™ (Applied Biosystems) and TMT® (Proteome Science), and applied them to an in-gel digest containing 2 proteins in order to distinguish the expression profile of the different proteins present in the spot (Fig. 1).

Methods

To optimize the labeling procedure and determine the quantitative accuracy, different amounts of a protein mix (100-360ng/protein) were loaded onto a 1D SDS-PAGE and stained using silver staining. Paired protein bands were excised and in-gel digested using trypsin. The extracted peptide samples were labeled with either iTRAQ™ or TMT® labels and analysed on-line by nano-LC MS/MS. The MS/MS spectra were analyzed using Mascot 2.2 (Matrix Science). The technique was validated on a second SDS PAGE construct. We therefore mimicked co-migration by combining bands containing the same amount of one protein (10ng) with bands containing different amounts of another protein (10-120ng) and compared those pooled spots one to one.

Results

45 and 35 gel band ratios were tested for iTRAQ™ and TMT® respectively. Technical variation in the measured ratios was normalized by applying the correction factor calculated to result in a 1:1 ratio in trypsin concentration. A 1.3 (or 0.77) ratio of expression was defined as the required minimal difference for adequate quantitation using this approach. Outside the threshold limits a 12% and 8% error rate was found for iTRAQ™ and TMT® respectively for correctly defining up- or downregulation. Both technical variation and the normalization using the trypsin ratio contribute to this margin of error.

When we mimicked co-migration however, technical variation became irrelevant and no normalization was needed, as only the difference between both proteins in each spot is relevant. This way, all predicted differences were measured correctly. As only 2 different labels are required, TMT® was considered the best candidate for routine application of this approach.

Innovative aspects

We have developed a straightforward approach to determine the relative contribution of two co-migrating proteins to the difference in spot intensity found during 2-D PAGE analysis.

References

(1) Gygi, S.P., *et al.*, *Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology.* Proc Natl Acad Sci U S A, 2000. **97**(17): p. 9390-5.

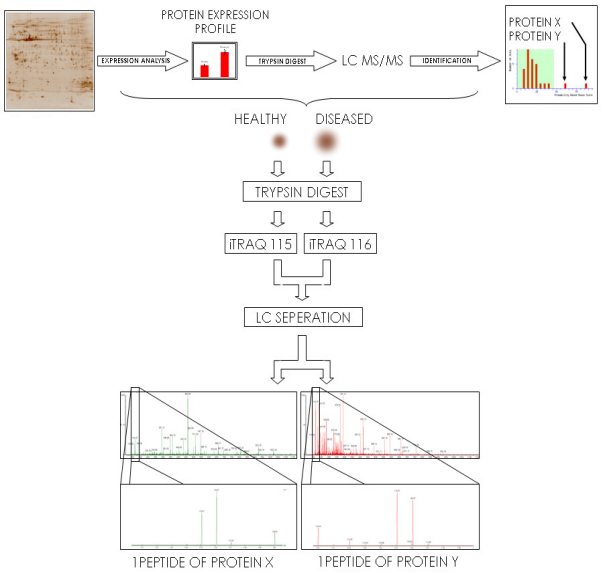

Figure 1. Workflow for resolving the expression profile of two co-migrating proteins present in a spot differentially displayed between two sample groups.