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A ROBUST, HIGH-THROUGHPUT METHOD FOR QUANTITATIVE, IN-DEPTH ANALISYS OF PROTEOMES

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Quantitative proteomics plays an increasingly important role in biological and medical research. MS-based quantitative proteomic methods have proven to be a very robust alternative to the well-established gel-based techniques. However, reliable MS-based high-throughput quantification is still a challenge. Here, we present a high-throughput quantitative proteomics method that allows a rapid, robust and deep analysis of proteomes. The samples are applied to conventional SDS-PAGE gels, but the run is stopped as soon as the front enters the resolving gel, so that the whole proteomes become concentrated in the stacking gel. The concentrated gel bands are subjected to trypsin digestion in the presence of the detergent CYMAL, and the resulting peptides are labelled with $^{16}O/^{18}O$. The mixture of labelled and unlabelled peptides is separated into 24 fractions by IEF using an OFF-Gel electrophoresis unit, and the fractions are analyzed, under high peptide loading conditions, by RP-HPLC-MS/MS using a linear ion trap LTQ MS. On average, more than 500 unique peptides were identified per fraction, with a peptide redundancy in different fractions of less than 2, resulting in the identification of more than 6,000 peptides at a FDR of 5%, corresponding to more than 2,300 proteins. Analysis of the data using QuiXoT demonstrates that the OFF-Gel technology does not affect O^{18} peptide labelling. Besides, analysis of the data using a previously proposed statistical model indicated that the variances at the scan, peptide and protein levels are similar to those encountered using in-solution digestion and SCX peptide fractionation. The method has been applied to the analysis of total protein extracts from brain, liver and endothelial cell cultures, and also of membrane proteomes such as rat heart mitochondria.