

Large-Scale Proteomics for Understanding Biological System



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Studying biological system and its perturbations requires information on organization and dynamics of proteome. This is commonly carried out by LC-MS/MS based proteomics, in which sample preparation and quantitative analysis of the spectrometric data play an essential role.

Filter Aided Sample Preparation (FASP) is a flexible and efficient way of processing protein extracts for bottom-up proteomic analysis. The method repurposes centrifugal ultrafiltration concentrators for depletion of detergents, protein digestion and isolation of pure peptide fractions. FASP can be used for protein cleavage with different proteinases either using single enzymes or in a mode of consecutive multienzyme digestion (MED-FASP). The FASP methods are useful for processing of samples ranging in their sizes from sub-microgram to several milligram amounts of total protein. Therefore, FASP is applicable for analysis of minute amounts of laser capture micro-dissected tissue

as well as for generation of large amount of peptides required for affinity enrichment of phospho- or glycopeptides.

Total Protein Approach (TPA) is a label- and standard-free method for absolute protein quantitation of proteins using large-scale proteomic data. The method relies on the assumption that the total MS signal from all identified proteins in the dataset reflects- in a 'biochemical sense'- the total protein and the MS signal from a single protein corresponds its abundance in the studied sample. The method offers a straightforward way to quantify thousands of protein per sample at their specific concentrations. A related method, the 'Proteomic Ruler' enables conversion of the protein abundance data calculated by TPA to compute numbers of protein copies per cell. TPA and the 'Proteomic Ruler' are powerful tools for studying dynamics of cell architecture.

The most relevant publications:

1. Universal sample preparation method for proteome analysis. Wiśniewski JR, Zougman A, Nagaraj N, Mann M. *Nat Methods*. 2009; 6:359-62.
2. A "proteomic ruler" for protein copy number and concentration estimation without spike-in standards. Wiśniewski JR, Hein MY, Cox J, Mann M. *Mol Cell Proteomics*. 2014 Dec;13(12):3497-506.
3. High recovery FASP applied to the proteomic analysis of microdissected formalin fixed paraffin embedded cancer tissues retrieves known colon cancer markers. Wiśniewski JR, Ostasiewicz P, Mann M. *J Proteome Res*. 2011; 10:3040-9.
4. Quantitative Analysis of Human Red Blood Cell Proteome. Bryk AH, Wiśniewski JR, *J Proteome Res*. 2017 Aug 4;16(8):2752-2761.
5. Extensive quantitative remodeling of the proteome between normal colon tissue and adenocarcinoma. Wiśniewski JR, Ostasiewicz P, Duś K, Zielińska DF, Gnad F, Mann M. *Mol Syst Biol*. 2012;8:611.