Technology Offer

Protein Epitope Signature Tags (PrESTs) for SILAC-based absolute protein quantification

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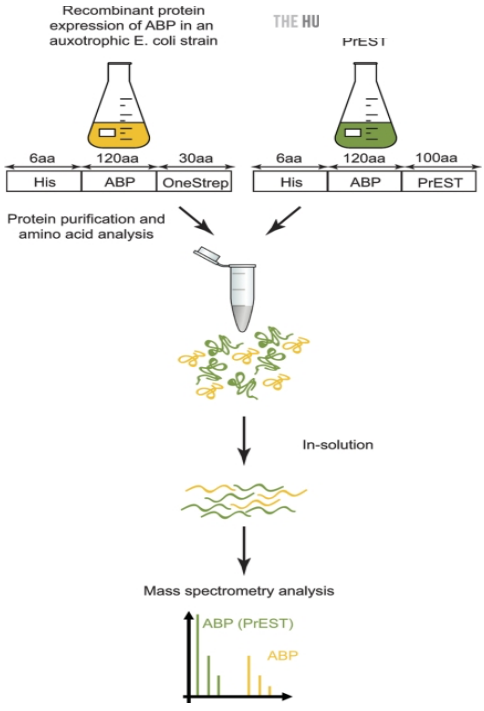
Direct quantification of the absolute amount of multiple proteins in one a SILAC experiment using Protein Epitope Signature Tags (PrESTs) which consist of a short and unique region of the protein of interest as well as purification and solubility tags and are expressed recombinantly in *E. coli*

Background

Mass spectrometry-based proteomics increasingly relies on absolute quantification which is technically challenging and can only be performed accurately for a single or a small number of proteins at a time. Current methods like AQUA, QConCAT, PSAQ, absolute SILAC and FlexiQuant generally require recombinant expression of labeled protein standards and quantify the endogenous protein of interest by the heavy to light ratios to a defined amount of the labeled counterpart spiked into the sample. However, each of the approaches comes with certain drawbacks. PSAQ, absolute SILAC and FlexiQuant rely on the production of full length proteins, which limits throughput and generally restricts these methods to soluble proteins. AQUA peptides are currently relatively expensive, especially when many peptides or proteins need to be quantified, and suffer from quantification uncertainties that are introduced due to spiking in of the peptide standard at late stages in the workflow. QconCAT does not automatically correct for protein fractionation effects or digestion efficiency in the native proteins vs. the spiked concatamers of proteotypic peptides.

Technology

Our scientists from the Max Planck Institute of Biochemistry have advanced the absolute SILAC approach to overcome any of the above-mentioned drawbacks. They employed short Protein Epitope Signature Tags (PrESTs) which consist of a short and unique region of the protein of interest as well as purification and solubility tags and are expressed recombinantly in *E. coli*. First, a highly purified, SILAC-labeled version of the solubility tag is quantified and used to determine the precise amount of each PrEST by its SILAC ratios. The PrESTs are then spiked into cell lysates and the SILAC ratios of PrEST peptides to peptides from endogenous target proteins yield their cellular quantities. The procedure can readily be multiplexed, as demonstrated by simultaneously determining the copy number of 37 proteins in HeLa cells. Direct quantification of the absolute amount of single proteins is possible via a SILAC experiment in which labeled cell lysate is mixed both with the heavy labeled solubility tag and with the corresponding PrEST. The SILAC - PrEST combination allows accurate and streamlined quantification of the absolute or relative amount of proteins of interest in a wide variety of applications.



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Patent Information

Three patents were granted in EP and US (EP2694556A1, EP2767834A2, US9063149B2).

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