

ANALYSIS OF LOW ABUNDANT MEMBRANE PROTEINS USING THE GEL-FREE PROTEIN SEQUENCE TAG (PST[®]) TECHNOLOGY

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About 25% of open reading frames in fully sequenced genomes are estimated to encode integral membrane proteins (1) that represent valuable targets for drugs. However, the global analysis of membrane proteins has been proven to be problematic e.g. because of their very amphiphilic nature and their low abundance. Here, we show that the Protein Sequence Tag (PST[®]) technology combined with an efficient sample preparation is a powerful method to perform protein analysis of highly enriched membrane fractions. PST is a gel-free proteomics tool for the analysis of proteins, which relies on a "sampling" strategy by isolating N-terminal peptides (considered as protein sequence tags) from Cyanogen bromide cleaved proteins (2). The identification of these N-terminal peptides is based on LC-MS/MS. The effectiveness of the technology is demonstrated for a membrane fraction, which was isolated from crude mitochondria of yeast after alkaline sodium carbonate treatment. The PST approach performed on this fraction analysed 148 proteins of which 80% are identified as membrane proteins. Thus, the removal of soluble proteins by sodium carbonate treatment increased the representation of membrane proteins in the preparation. More interestingly, among these membrane proteins 61% are predicted to be of low abundance. These encouraging results are an important step towards the development of a quantitative PST approach for the differential display of membrane protein analysis.

- (1) Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E. L. (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J. Mol. Biol.* 305, 567-580
- (2) Kuhn, K., Thompson, A., Prinz, T., Müller, J., Baumann, C., Schmidt, G., Neumann, T., and Hamon, C. (2003) Isolation of N-terminal protein sequence tags from cyanogen bromide cleaved proteins as a novel approach to investigate hydrophobic proteins. *J. Prot. Res.* 2, 598-609