

## **Exploiting the membrane subproteome. A shotgun peptide immobilised pH gradient – isoelectric focusing approach\***

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Membrane proteins play an important role in pathogenesis and are one of the most exploited targets for therapeutic intervention. Proteomics is contributing to improving the understanding of many disease processes at a molecular level. Despite this, traditional proteomic methods used to analyse and identify membrane proteins remain a significant bottleneck where there has been slow progress.

Overcoming physicochemical limitations dictated to by the hydrophobic nature of the vast majority of membrane proteins is a key factor to enhancing the analysis of this repertoire of proteins. We address this inherent limitation by employing a shotgun proteomic approach. An established form of this technique involves tryptically digesting a complex mixture of proteins and separating the generated peptides over single or multidimensional liquid chromatography mass spectrometry. Here, we present preliminary data obtained from analysing rat liver membrane proteins utilising a peptide immobilised pH gradient - isoelectric focusing coupled with this shotgun proteomic approach.

Preliminary analysis of a total of 24 fractions of peptides focused over a pH range of 3-10 yielded an identification of 2957 non redundant peptides, corresponding to a total of 761 unique proteins with a false positive rate of 1%. 51 of these proteins have a GRAVY score of > +0.3, validating the suitability of the approach for hydrophobic proteins that are often found to be refractory to analysis by 2DE gels. Some examples of the rat liver membrane proteins identified using this approach include putative transferrin receptor protein 2 (TfR2), solute carrier family proteins and many cytochrome P450 genetic variants, to name but a few. This study has illustrated that the majority of rat liver tryptic peptides focused within the pH range of 3.3-5.2. Further in-depth analysis of the peptides within this narrow IPG strip pH range will facilitate better peptide resolution and subsequently enable further coverage of the membrane subproteome.

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