Keeping Proteins in Solution: Separation of Membrane Proteins Using Free-Flow Electrophoresis

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Membrane proteins perform some of the most important functions in the cell, such as the regulation of cell signaling through surface receptors, cell-cell interactions and ion transport across the membrane. In drug discovery the membrane proteins account for more than 70% of all known pharmaceutical drug targets and 25% of all experimental and marketed drugs target one class of membrane proteins, the G-protein coupled receptors. Despite the importance of membrane proteins they have so far not been characterized to the same extend as soluble proteins. Membrane proteins are embedded in the hydrophobic core region of the membrane bilayer by interaction with the hydrophobic lipids phase. They consist of hydrophobic and hydrophilic regions and it is their amphiphilic nature that makes them difficult to investigate.

Two-dimensional gel electrophoresis in combination with solubilizing agents such as urea/thiourea and nonoionic/zwitterionic detergents has been employed for the separation and identification of membrane proteins but with limited success. The obstacles remain the hydrophobic nature of the membrane proteins and their tendency to precipitate at their isoelectric point. In addition, extraction of peptides following the in-gel digestion procedure remains difficult with low recovery rates due to the hydrophobic nature of the generated peptides.

Free-flow electrophoresis (FFE) provides an alternative separation methodology to two-dimensional gel electrophoresis that is purely liquid based and not limited in the applicable pH or size range. So far, proteins separations using FFE have been carried out in the isoelectric focusing mode rendering this method less useful for membrane proteins. To overcome this limitation we developed a novel separation mode for FFE called interval zone FFE. In contrast to the isoelectric focusing mode, the separation is carried out at a constant pH relying on the net protein charges. The applied pH of the separation buffers is selected such that it is different from the isoelectric point of the proteins to be separated thereby maintaining proteins in solution that would otherwise precipitate. We demonstrated the usefulness of this novel FFE mode by applying it to different membrane preparations under native or denaturing conditions. Under denaturing conditions the addition of detergents to the separation buffers was not required to

maintain the proteins in solution. This enabled an easy and straightforward coupling to liquid chromatography and tandem mass spectrometry.