

## Separation of Membrane Proteins

**T. Rabilloud**

IRTSV/LBBSI, CEA Grenoble, 17 rue des martyrs, 38054 Grenoble cedex 9; CNRS UMR 5092 Thierry.Rabilloud@cea.fr

The analysis of membrane proteins is plagued by several difficulties. The first problem arises from the very definition of what is a membrane protein. Biological membranes are made of lipids, and several modes of association of proteins to lipids are encountered. These can be direct interaction modes, such as transmembrane helices, beta barrels or anchoring lipids. But these can also be indirect interaction modes, e.g. in the various subunits of a protein complex partly anchored in the membrane.

As a matter of facts, the so-called membrane preparation contain an important amount of proteins that are not directly associated with the membrane lipids, and it is quite often difficult to figure out what is a real indirect association or a simple artefact linked to the biochemical preparation.

The second difficulty that arises comes from the very nature of intrinsic membrane proteins, i.e. proteins in which (a) polypeptide segment(s) drive(s) the association of the protein to the lipids. The structuration of these proteins usually shows lipophilic domains and hydrophilic domains. This means in turn that keeping the complete protein in solution is a difficult task. Most often, membrane proteins are indeed not soluble into organic solvents, but are soluble only in micellar environments, made of surfactants dissolved in water, and which can be seen as a dispersion of a lipid-like environment in water. The membrane proteins are often exquisitely sensitive to details in the surfactants structure, and their solubility is often very limited.

These solubility problems are of course maximized when methods using the isoelectric point are used, as the pI is by definition a solubility minimum. Thus, the classical 2D PAGE separation strategies usually perform rather poorly with intrinsic membrane proteins, although discrete but limited success has been obtained with special surfactants-chaotropes cocktails. This has led to development of alternate approaches, (e.g. using double zone electrophoresis separations) which perform better as far as solubilization is concerned but offer in turn a much lower resolution.

These various problems and limitations, both in sample preparation and in protein separation per se, will be discussed here, as well as possible ways for improvement.