

## TARGETED PROTEOMICS: A POWERFUL APPROACH PROVIDING NEW INSIGHTS IN BIOLOGY.

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The introduction of ultrathin gels (< 0.5 mm) polymerized on cellophane as support (Görg et al., *Anal. Biochem.* 1978) - a defiance to the traditional tube gels for disc electrophoresis and IEF with carrier ampholytes as well as to the conventional 2 mm thick polyacrylamide gels- improved pattern resolution and reproducibility, whereas the development of ultrathin pore gradient gels cast on plastic backing for horizontal SDS electrophoresis (Görg et al., *J. Biochem. Biophys. Methods.* 1980) paved the way for the realisation of the "Immobiline\* Project" (Bjellqvist *et al.*, *J. Biochem. Biophys. Methods.* 1982, 6, 317-339). However, our 2-DE pattern we included in this article was, more or less, only a proof of principle. It was only the beginning of a long journey of stepwise improved 2-DE protocols we developed in our laboratory and summarized in different reviews (*Electrophoresis* 1988; 2000; 2009, *Proteomics* 2004). Milestones were the *development and design of the IPG DryStrip* (3mm wide, 0.5 mm thin, 7-24 cm long, cast on plastic backing; Görg et al., *Electrophoresis* 1988) and the "*reduction-alkylation equilibration protocol*" of IPG strips after IEF for the efficient transfer of proteins from first to second dimension. The protocol of 2-DE with IPGs has been constantly refined, *e.g.* by the generation of tailor-made IPGs with different pH intervals from the acidic to the basic extremes (pH 2.5-12), and extended separation distances for improved resolution. A historical outline from the technical difficulties encountered during the development of 2-DE with IPGs, to the establishment of the actual "standard protocol" will be given, as well as the modified procedures for the separation of very acidic, very alkaline, low abundance and hydrophobic proteins.