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Characterization of the gel protein interaction in polyacrylamide gels during electrophoresis separation in proteomics by physical and spectroscopic measurement

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Polyacrylamide gels were first introduced as support for electrophoresis techniques in 1959 and, they still represent the main separation tool in biology. The most routinely used gels are based on the SDS-PAGE method (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) in which proteins are denatured and solubilized by the anionic detergent. This SDS-PAGE technology remained stable for decades. However the separation is also very sensitive to the exact nature of the chemicals in the gel. Very few studies have been devoted to the understanding of the local interactions between the gel, the migration solvent and the proteins that ultimately define the quality of separation. Our group developed innovative solutions to control the pore size of the gel by incorporating Dextran in the gel monomer solution which serves as porogen. This implementation allowed obtaining superior protein separation qualities (higher spot resolution) compared to commercial gels. We have also developed original gels for separation of membrane proteins by introducing an hydrophobic monomer N,N'-dimethylacrylamide. Today there is no method to correlate the technical preparation of gels, and their monomer composition with the chromatographic properties. Indeed electrophoresis gels are highly cross linked; this ascertainment greatly reduces the number of techniques that can be used.In this study the gels were characterized both for their global properties by rheometry and Differential Scanning Calorimetry (DSC) and for their local properties by liquid Nuclear Magnetic Resonance (NMR), Electron Spin Resonance (ESR) and fluorescence spectroscopy in function of SDS content, crosslinker percentage and added hydrophobic monomer content. Rheometry gave us access to viscosity of the gel solution and its variation with SDS concentration. DLC allowed determining the temperature of freezing of the water in contact with the gel. We followed the signal displacement of adapted probes to localize by ESR or NMR model molecules on the gel or in solution and finally, we studied the same phenomenon by fluorimetry using model peptides.All these data will be integrated together to afford a comprehensive model of the acrylamide gel interactions during protein separation by electrophoresis. The authors thank N. Touati, H. Vezin for ESR measurements, M. Bria and X. Trivelli for NMR measurements, C. Pierlot for Rheometry experiments, U. Maschk for recording the DLC and J. Albani for fluorimetry investigations.

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