

OF8 Isolation and characterisation of protein complexes using blue native polyacrylamide gel electrophoresis (BN PAGE) and mass spectrometry.

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BN PAGE is an ideal way to isolate intact protein complexes for MS analysis

The concept of replacing SDS in polyacrylamide gel electrophoresis with mild binding agents to impart an overall negative charge when binding to proteins yet not denature them, has been around for some time [1]. However this concept of blue native gels (BN PAGE) has perhaps not been realised to its full potential.

Both soluble and membrane-bound protein complexes from a variety of plant animal and microbial sources were isolated as intact entities using native gel electrophoresis with Coomassie blue G-250 as the binding agent. Characterisation of the isolated bands following trypsin digestion and tandem mass spectrometry by FT-ICR MS revealed that the protein complexes exhibited expected stoichiometries for their respective subunit compositions. Sub-stoichiometric amounts of other known interacting factors were also observed.

The approach was extended to the analysis of small molecular weight ligand binding proteins where passively bound volatile ligands could be isolated still associated with their binding partners. Ligands were examined by capillary GCMS following solid phase microextraction of the headspace from gel slices that had been proteolytically digested to release bound compounds.

[1] H. Schagger, G. von Jagow, *Anal. Biochem.* 1991, 199:223-231.