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Blue-native polyacrylamide gel electrophoresis: A useful tool for study of oligomeric state/native molecular weight of mitochondrial membrane protein complexes

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Blue-Native Polyacrylamide Gel Electrophoresis (BN-PAGE) was originally described by Schägger and von Jagow as a technique used for fractionation of enzymatically active membrane protein complexes. Prior to electrophoretic separation, which is carried in the presence of the anionic dye Coomassie Blue G-250, protein complexes are extracted from the membrane using non-ionic detergent (digitonin, dodecyl maltoside, Triton X-100). Subsequently, Coomassie Blue G-250 is added to the solubilized protein complexes. Equilibrium between protein-associated detergent-lipid micelle and protein-bound G-250 dye is established and the samples are ready to load onto the gels. From this moment on, the electrophoretic run closely resembles that of conventional denaturing PAGE. We have used this technique coupled to second dimension denaturing (SDS) PAGE and subsequent western blotting to study assembly state of mitochondrial oligomeric protein complexes I and IV. We have identified several assembly intermediates of the complexes ranging from approx. 10 to 950 kDa. Very recently, we used BN-PAGE together with second dimension denaturing run successfully for assessment of native molecular weight/oligomeric state of mitochondrial protease YME1L as well as mitochondrial ATPase LACE1. To summarize, when employed in conjunction with preceding protein mixture prefractionation and subsequent protein identification using either western blotting or mass spectrometry, this relatively simple and straightforward technique still proves very useful when studying mitochondrial membrane protein complexes.

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