Blue Native electrophoresis to study mitochondrial and other

2.3. Second dimension

A lane is cut out of the first-dimension gel with a razor blade (Fig. 1). The strip is rotated through 90 , placed on a glass plate, and incubated with a dissociating solution (1% SDS and 1% 2-mercaptoethanol), for 1 h at room temperature. Excess dissociating solution is drained away using a filter paper and the glass plates are assembled according to the manufacturer's instructions. Careful removal of dissociating solution is essential as 2-mercaptoethanol inhibits gel polymerization. Often the first-dimension spacers are thicker (e.g., 1.5 mm) than those of the second-dimension gel (e.g., 0.75 mm). Thus, the first-dimension strip is compressed between the glass plates, making it relatively easy to cast the second-dimension separation gel without contact occurring between the two. After polymerization, the stacking gel is poured around

single entity [12] and that it again retains ATP hydrolysis activity [13]. To visualize OXPHOS complexes in a quantitative way Zerbetto and colleagues exploited the fact that protein complexes retain enzyme activity on BN-PAGE [14]. The authors treated gels with solutions for histochemical staining of specific complexes. The use of these histochemical staining methods made it possible to combine enzyme activity with the mobility of the complex in the gel. A major advantage of the method is that nonspecific activities do not interfere, because they are unlikely to have the same size and mobility as the complex of interest. Where a complex has an altered mobility the e ect on catalytic activity can be readily ascertained (Fig. 2). For instance, when F_1 dissociates from the F_1, F_0-ATPase it maintains its dephosphorylating activity (Fig. 2).RROq[AEO)Fqq–A!+In-)[zig.wEU[;)FzactivitwE[)[zUqUF11)[asurOFhwE[)1)[zqOFwE[ains,Forde

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identification of a spot on a gel. Great sensitivity and