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Clear Native Gel Electrophoresis for the Purification of Fluorescently Labeled Membrane Proteins in Native Nanodiscs

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Native gel electrophoresis is widely used to separate and characterize proteins. However, high-resolution clear native electrophoresis (CNE) often suffers from membrane protein aggregation and the lack of suitable molecular weight markers. Here, we present a novel approach that combines charged polymer-encapsulated nanodiscs with fluorescence correlation spectroscopy (FCS) to overcome both challenges. Membrane proteins are first extracted using Glyco-DIBMA, a negatively charged amphiphilic copolymer. The spontaneously formed nanodiscs incorporate fluorescently labeled target proteins within a native-like lipid bilayer, as confirmed by FCS. These nanodiscs are then subjected to detergent-free CNE. As the number of protomers increases, nanodiscs grow larger and migrate further in the gel due to their increased charge density. FCS of resolubilized gel bands confirms that nanodiscs remain intact. Moreover, membrane proteins do not aggregate, as evidenced by their fluorescent brightness and diffusion times. Importantly, the oligomeric state of membrane proteins can be deduced from the brightness per nanodisc. Since purified membrane proteins remain embedded in a native-like bilayer and are never exposed to detergent, they are immediately suitable for downstream structural and functional studies.