FUNCTIONALIZATION OF SMA NANODISCS

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ABSTRACT

The integration of membrane proteins into biosensors or biophysical assays is challenging due to difficulties associated with their purification and conjugation. I will present newly developed functionalization procedures to interface membrane proteins with dyes or surfaces, via functionalization of lipid nanodiscs. Nanodiscs are nanometer-sized discoidal phospholipid bilayers that are surrounded by an amphipatic polymer, such as the membrane scaffold protein or styrene-maleic acid (SMA) co-polymer. Membrane proteins embedded in lipid nanodiscs maintain their membrane-integrated state in this soluble complex. SMA co-polymers are highlighted as agents for detergent-free purification of membrane proteins, which can solubilize membrane proteins in presence of their native lipid membrane environment. This approach considerably eases purification of membrane proteins, but does not enable their detection or immobilization as such. To facilitate conjugation of SMA-nanodiscs to surfaces, nanoparticles and fluorophores, we modify the SMA polymer with cysteamine. To accomplish this, we exploit the reactivity of maleic anhydride moieties in SMA towards amines to equip the polymer with a sulfhydril group (SMA-SH). This sulfhydryl group is then modified with thiol-reactive probes, such as maleimide derivatives of fluorophores and biotin. We find that SMA-SH enables the functionalization of membrane proteins with a variety of different probes, while not requiring any mutation or chemical modification of the protein itself. We anticipate that this versatile approach will find application in membrane protein purification, in biosensing, as well as in a wide range of biophysical assays, such as single-molecule TIRF measurements, AFM, optical or magnetic tweezers.