NMR, EM and Computation for Membrane Proteins Studies in Nanodiscs

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Membrane proteins are key to many biological processes. However, structural and functional studies are hampered by difficulties of using suitable membrane surrogates that mimic the natural membrane environment. We have developed procedures for formation of covalently circularized phospholipid nanodiscs that are small patches of phospholipid bilayer surrounded by membrane scaffolding proteins MSPs originally derived from apolipoprotein A1 [1]. Initial attempts to insert integral membrane proteins into nanodisc for NMR studies resulted in systems that could be studied with NMR and electron microscopy; however, the nanodiscs were heterogeneous in size and the number of membrane proteins inclosed[2]. To overcome this problem we developed procedures for covalently circularizing scaffold proteins. This results in nanodiscs of exactly defined diameters. The nanodisc size can be tuned to the size and number of membrane proteins enclosed. Stoichiometry of complexes can be precisely established with a DNA-tethering approach [3]. The membrane proteins are suitable for NMR and EM studies. We have developed methods for ^{15}N-detected TROSY experiments that open avenues for studies of much larger systems [4]. Data acquisition is facilitated by using non-uniform sampling (NUS)[5-8] and processing methods based on the iterative soft thresholding principle (hmsIST)[9]. The research is suitable for interfacing with centers focused on electron microscopy and advanced data processing.

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