Lipid bilayer nanodiscs: a promising system for in-solution studies on GPCR binding

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G protein-coupled receptors (GPCRs) are an important family of 7-transmembrane domain proteins responsible for the activation of several signal transduction pathways in virtually all organ systems [1]; for this reason, GPCRs represent the molecular targets for almost 40% of all pharmaceuticals currently used in clinical practice and marketed, and are involved in the treatment of a wide range of pathological conditions. Due to the complex mechanisms leading to the modulation of GPCR activity, an impressive volume of medicinal chemistry research has been devoted to the investigation of the biochemical pathways mediated by GPCRs, aiming at the discovery of new potential targets and the development of highly selective and potent drugs. The development and application of biophysical and spectroscopic techniques to the *in vitro* investigation of GPCR modulation is, however, hampered by the low solubility of these targets in aqueous media. The emerging technology of lipid bilayer nanodiscs (LBNs) was therefore investigated to determine its suitability for in-solution studies on GPCR binding.

LBNs are highly soluble, nano-scale lipid bilayers which can incorporate single, fully functional membrane proteins through simple chemical self-assembly [2]. Within this project, LBNs were prepared mimicking the native environment of eukaryotic cell membranes by using membrane scaffold protein MSP1E3D1 and phospholipids POPC and POPG. The LBN self-assembly was carefully investigated by a combination of several techniques, including size exclusion chromatography (SEC), dynamic light scattering (DLS), transmission electron microscopy (TEM) and circular dichroism (CD) spectroscopy. The critical parameters for a controlled and reproducible self-assembly were identified and optimized, allowing to develop a new procedure for the preparation of stable LBNs. According to the results, LBN systems should be a suitable tool in the development of new methods for in-solution studies on GPCRs based on isothermal titration calorimetry (ITC) [3] and CD spectroscopy [4].

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