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Victoria Schmidt, James N. Sturgis

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### **ACCEPTED MANUSCRIPT**

# Modifying styrene-maleic acid co-polymer for studying lipid nanodiscs

Victoria Schmidt and James N. Sturgis

LISM UMR 7255, CNRS and Aix-Marseille University, 31 Chemin Joseph Aiguier, 13402 Marseille cedex 20, France

#### Abstract

Recently, styrene-maleic acid copolymer lipid nanodiscs have become an increasingly popular tool for the study of membrane proteins. In the work we report here, we have developed a novel method for the efficient preparation of labeled nanodiscs, under chemically mild conditions, by modification of the hydrolyzed styrene-maleic acid copolymer. This protocol is designed to be easily accessible to biochemistry laboratories. We use this procedure to prepare various fluorescent nanodiscs labeled with different fluorophores. By studying the development of Förster resonance energy transfer, we demonstrate the rapid exchange of co-polymer between nanodiscs. This demonstration, in conjunction of previous work, indicates that the lipid nanodiscs prepared using this polymer are very dynamic structures with rapid exchange of the different components.

Keywords: SMALP, Membrane Protein, Nanodisc

#### 1. Introduction

The *in vitro* study of membrane proteins can be particularly challenging [1]. This challenge arises from various steps in a typical protocol, that includes over-expression, membrane isolation, solubilization and purification. During solubilization detergents are typically used to extract the proteins from their native lipid environment, and to replace the lipids with detergent molecules. Finding an appropriate detergent is complex, and the delipidation can often result in loss of activity or denaturation [2]. The difficulties associated with using detergents have led to the development of several alternative strategies for maintaining membrane proteins in solution including

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