

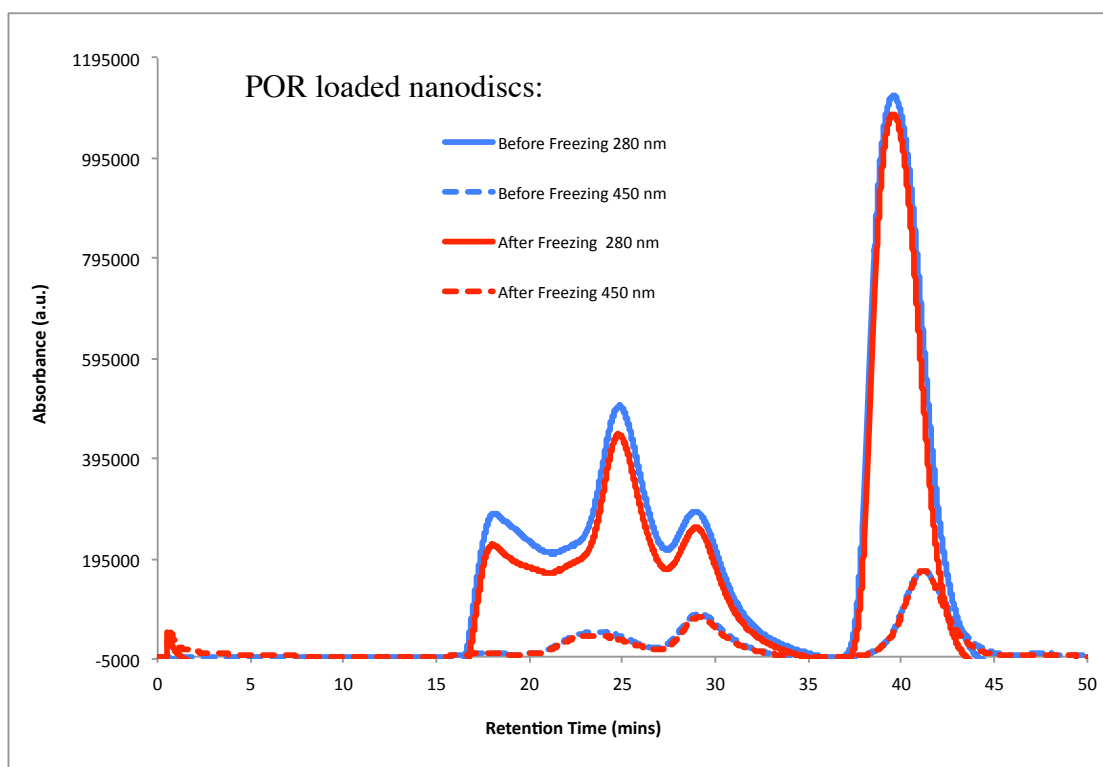
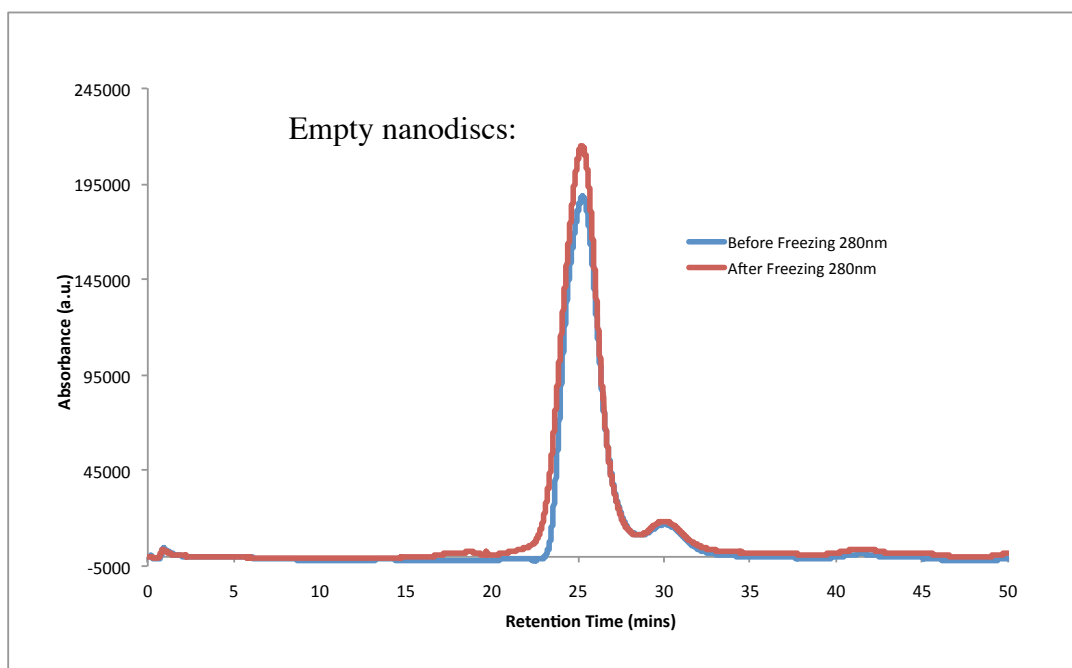
# **Nanodisc Films for Membrane Protein Studies by Neutron Reflection: Effect of the Protein Scaffold Choice – Supporting Information**

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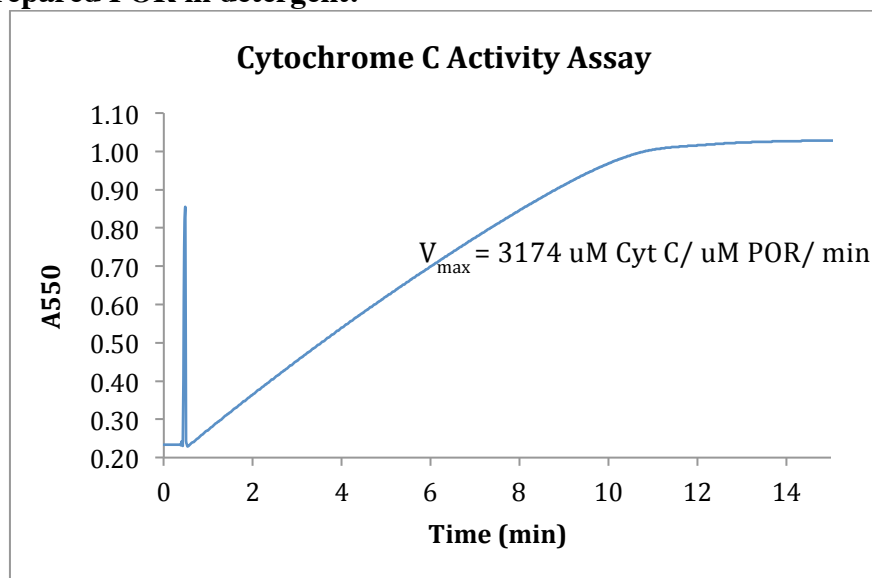
HPLC chromatograms (Figure S1) show the structural integrity of both empty and POR loaded MSP1E3D1 based nanodiscs before and after freezing to -80 °C. Nanodiscs reconstitution (Empty and POR loaded) was made and the nanodiscs preparation was split in two. One fraction was subject to -80 C, while the other one was kept at 4 °C. Then, these two nanodisc preparations were purified by HPLC. Re-running an already purified nanodiscs sample is not possible due to the high dilution in the sample. We are quite limited with the protein amounts and we cannot afford to use all the samples already purified to undergo a second HPLC run. We do not believe in concentrating the nanodiscs to avoid structural or functional distortions.

The functional integrity assessment of POR Nanodiscs that were frozen at -80° C was conducted by Cytochrome C Assay as in Ref <sup>1</sup>(Figure S2). Briefly, the time-dependent reduction of cytochrome-c was measured at 550 nm using a Perkin Elmer Lambda 800 Spectrophotometer. The reaction was performed in a 1ml cuvette containing 1.2 nM POR and 50 µM of cytochrome C. Reduction of cytochrome c was initiated by addition of 1 mM NADPH. The slope in the linear region of the curve was used to determine the POR activity. The Vmax of POR Nanodiscs after freezing was found to be 3174 uM Cyt C/ uM POR/ min which is comparable to the previously published values of ~3500 µM cyt-c/µM POR/Min (see Ref <sup>2</sup>) for freshly prepared POR in detergent.

**Figure S1. HPLC chromatogram for empty and POR loaded MSP1E3D1 nanodiscs before and after freezing to -80 °C.**



**Figure S2. Cytochrome C functional assay in POR loaded MSP1E3D1 nanodiscs after freezing to -80 °C. The obtained V<sub>max</sub> is comparable to the previously published values of for freshly prepared POR in detergent.<sup>2</sup>**



**References:**

1. Guengerich, F. P.; Martin, M. V.; Sohl, C. D.; Cheng, Q. Measurement of cytochrome P450 and NADPH-cytochrome P450 reductase. *Nature protocols* **2009**, *4*, 1245-1251.
2. Laursen, T.; Naur, P.; Møller, B. L. Amphipol trapping of functional CYP system. *Biotech Appl Biochem* **2013**, *60*, 119-127.