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Production of the Na^+/Ca^{2+} Exchanger from *Methanococcus janaschii* for NMR Studies

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INTRODUCTION. Na^+/Ca^{2+} exchangers (NCX) are antiporters that use the Na^+ electrochemical gradient to export Ca^{2+}. The NCX may work in the reverse direction, importing Ca^{2+} and exporting Na^+, depending on the membrane potential and ion concentrations. The NCX contains 10 transmembrane helices (TM). **OBJECTIVE**: To express and purify the NCX from *Methanococcus janaschii* (NCX_Mj) in amounts suitable for recording NMR experiments. **MATERIALS AND METHODS**: NCX_Mj was cloned in fusion with a glycophorin-A TM helix, followed by the green fluorescent protein (GFP) and 9 histidines at the C-terminal end. Protein expression was performed in *E. coli*. Cell lysis was carried out using a French press. Membrane fraction and inclusion bodies were solubilized with n-dodecyl-β-D-maltoside (DDM). NCX_Mj was isolated by affinity chromatography on a Ni^{2+}-chelating resin, followed by gel filtration on a Superdex-200 column. **RESULTS AND DISCUSSION**: Mini-induction tests indicated that *E. coli* BL21(DE3) was the best strain for NCX_Mj super-expression. Detergents (Triton X-100 or DDM) were added to the lysate in order to solubilize the protein found in both the membrane and the inclusion bodies. Insolubilized debris was separated by ultracentrifugation. NCX_Mj was isolated in two steps of affinity chromatography followed by gel filtration. A band corresponding to the expected molecular weight of the fusion protein (~60 kDa) was observed in the SDSPAGE, the identity of which could be confirmed by fluorescence spectroscopy *in-gel*. Removal of the C-terminal GFP tag using the protease Human Rhinovirus 3C has to be tested.
CONCLUSION: The *E. coli* expression of a large transmembrane protein was successfully achieved. The isolation protocol has to be optimized in order to increase the yield and purity of the sample. The efficiencies of the solubilization and affinity chromatography steps seem to be dependent on the detergent used (Triton X-100 versus DDM).

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