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# Highly active membrane proteins produced in a cell-free expression system

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## Background

Dysregulation in membrane protein activity is often a primary step in human diseases. One of the major difficulties in the study of membrane proteins is to recover a large enough amount of recombinant proteins from the classical overexpression techniques. We have recently demonstrated the effectiveness of a cell-free expression system in production of membrane proteins. The membrane proteins are produced in a soluble form without the requirement of a denaturation step. Most of the membrane proteins tested in this expression system are with native-like conformation whatever the presence or not of lipid vesicles. Here, we examplify our protein expression system on membrane proteins from different origins: the mammalian gp91-phox protein, the large subunit of the flavocytochrome *b558*, the mitochondrial voltage-dependent anion channel, VDAC, and the chloroplastic outer envelope protein from pea, OEP24.

## Results

Gp91phox is an integrated transmembrane protein containing the catalytic activity of the reducing NADPH oxidase complex. Full length or truncated gp91-phox proteins were expressed in soluble forms in *E. coli* lysate in presence of non ionic detergents and in an oxidizing environment. Activities of the resulting proteins were tested in an electron transfer assay. Some of these gp91-truncated forms contained an enzymatic activity alike to the cellular counterpart. For these truncated proteins, scale-up experiments have been performed in order to use the purified proteins in crystallographic studies and structural analysis. VDAC and OEP24 membrane proteins were overexpressed at 230 μg/ml and 360 μg/ml respectively in our optimized cell-free protein synthesis system. Proteins are integrated in lipid vesicles in presence of detergents resulting in proteoliposomes. In presence of liposomes, OEP24 protein was recovered as dimers suggesting that OEP24 protein is functional. The OEP24 in proteoliposomes forms a cation-selective channel as measured by changes in suspension turbidity in presence of KCl or sucrose. Activity of recombinant VDAC proteoliposomes was directly tested on mammalian cells by adding the VDAC proteoliposomes on cells and by measuring the cytochrome c release and activation of apoptosis. Immunofluorescence studies using anti-VDAC and anti-His antibodies indicate that recombinant VDAC is targeted to the mitochondria.

## Conclusion

In conclusion, our recombinant technology to produce membrane proteins in their native conformations represents a new strategy for providing insights into functional and structural informations on membrane proteins.

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