Strategies for Membrane Targeting of Heterologous Proteins in *E. coli*

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**ABSTRACT**

The characterization of integral membrane proteins is limited not only by the reactivity in cellular expression, but also by the difficulty in isolating sufficient purified protein. Here we introduce a new strategy for improving the purification of integral membrane proteins. Our strategy involves an in vivo expression system for the assembly of integral membrane proteins. The expression system is coupled with an in vitro reassembly of the purified proteins, allowing for the isolation of purified recombinant proteins. The in vivo expression system is optimized to facilitate the expression of integral membrane proteins in a controlled manner, resulting in the production of high-quality recombinant proteins.

**RESULTS: PhoA fusion analysis**

Expression level of model PhoA fusion proteins - anti-PhoA blot

1) phoA
2) phO-A-CBD
3) subunit c-PhoA
4) subunit c(G23D)-PhoA
5) subunit c(L31F)-PhoA
6) b-actin protein marker

Conclusions:

PhoA fusions express at the highest levels (identical promoter, rbs) phoA-CBD-PhoA expression is most robust subunit c(G23D)-PhoA fusion is subject to in vivo proteolysis subunit c(L31F)-PhoA expression/stability is equal to wt subunit c-PhoA

PhoA provides a read-out for membrane assembly

**RESULTS: Functional expression of fused membrane protein**

Does pVII-CBD enable proper assembly of fused membrane proteins?

In vivo Assay for Expression of Functional Eukaryotic Membrane Protein

YidC is essential for E. coli viability

YidC-depletion strain J57131 Growth on L-glucose requires functional assembly of Oxa1p

Expression of pVII-CBD-Ent-OxaBHIS complements the loss of YidC in *E. coli*

Purification of pVII-CBD-Ent-OxaBHIS -analysis after nickel-NTA chromatography

Conclusion:

Identification of epitopes in anti-pVII and anti-CBD blots indicates pVII-CBD targeting peptide is resistant to undesired proteolysis

Membrane targeting peptides evaluated in this study:

wild-type pVII

pVII-CBD

subunit c (wild-type)

subunit c (G23D)

subunit c (L31F)