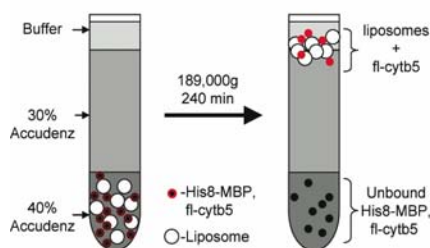


# Center for Eukaryotic Structural Genomics

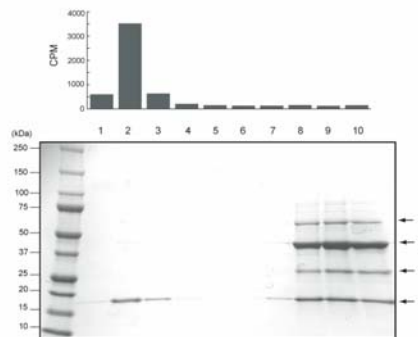
## Technology Dissemination Report

<b>CESG Tech Report No.</b>	016
<b>Title</b>	Structural Genomics Methods Applied to Production of the Monotopic Membrane Protein Human Cytochrome b5 and <i>in situ</i> Delivery to Liposomes
<b>Research Unit</b>	Protein Purification
<b>Authors</b>	Sobrado, P., Goren, M.A., James, D., Amundson, C.K., and B.G. Fox
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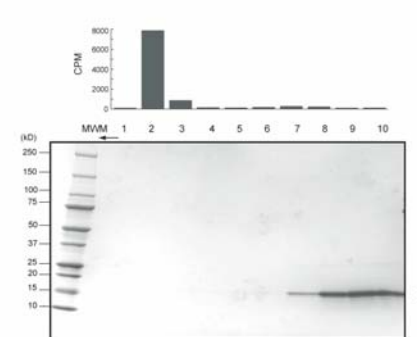
A. *In situ* delivery of full-length human cytochrome b5 to liposomes by TEV protease treatment of His8-MBP-fl-cytb5



B. SDS PAGE analysis of fl-cytb5 transferred to liposomes tracked by [<sup>14</sup>C]-phosphatidylcholine



C. Cytb5 lacking the membrane anchor does not transfer to the liposomes



### Summary

Fusion protein vectors developed for high-throughput protein expression as part of the Protein Structure Initiative have been investigated for use in the expression and stabilization of human cyt b5, a monotopic membrane protein that must be attached to the cellular membrane for function. Expression as a fusion to His8-maltose binding protein allowed expression of the full-length cyt b5 (fl-cytb5) as a fully soluble entity. Maintenance of the solubility in *E. coli* during the time course of expression was associated with high-level incorporation of protoporphyrin IX into the heme domain of the fusion protein. The fl-cytb5 could be liberated from the fusion by site-specific proteolysis, which permitted spontaneous incorporation into membrane vesicles. This work provides a convenient method for the production and high-yield *in situ* delivery of monotopic membrane proteins to lipid environments.

Publication:

- [1] Sobrado, P., Goren, M.A., James, D., Amundson, C.K., and Fox, B.G. (2007) A Protein Structure Initiative approach to expression, purification, and *in situ* delivery of human cytochrome b5 to membrane vesicles. *Protein Expr Purif*, 58(2):229-41.

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