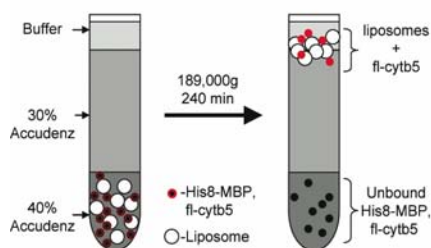


Center for Eukaryotic Structural Genomics

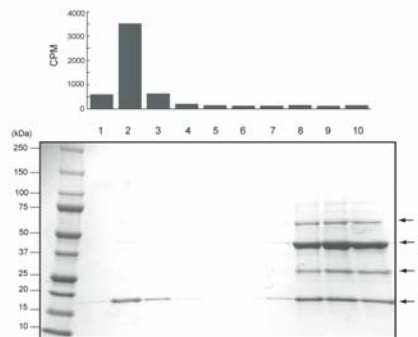
Technology Dissemination Report

CESG Tech Report No.	016
Title	Structural Genomics Methods Applied to Production of the Monotopic Membrane Protein Human Cytochrome b5 and <i>in situ</i> Delivery to Liposomes
Research Unit	Protein Purification
Authors	Sobrado, P., Goren, M.A., James, D., Amundson, C.K., and B.G. Fox
Primary Contact	bgfox@biochem.wisc.edu

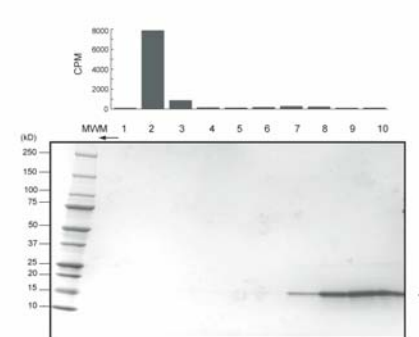
A. *In situ* delivery of full-length human cytochrome b5 to liposomes by TEV protease treatment of His8-MBP-fl-cytb5



B. SDS PAGE analysis of fl-cytb5 transferred to liposomes tracked by [¹⁴C]-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.

Acquiring the Technology	Contact Patrick Sweeny, Wisconsin Alumni Research Foundation prsweney@wisc.edu .
Other Acknowledgements	Also funded by NIH GM50853, B.G. Fox, PI.
Center for Eukaryotic Structural Genomics (CESG), University of Wisconsin-Madison Biochemistry Department, 433 Babcock Drive, Madison, WI 53706-1549; phone: 608.263.2183; fax: 608.890.1942; email: cesginfo@biochem.wisc.edu ; website: http://www.uwstructuralgenomics.org . This research funded by NIH / NIGMS Protein Structure Initiative grants U54 GM074901 and P50 GM064598.	