Center for Eukaryotic Structural Genomics

Technology Dissemination Report

CESG Tech Report No.	024
Title	Expression and Purification of Bacteriorhodopsin using Protemist XE with Detergents
Research Unit	Cell-Free Protein Production
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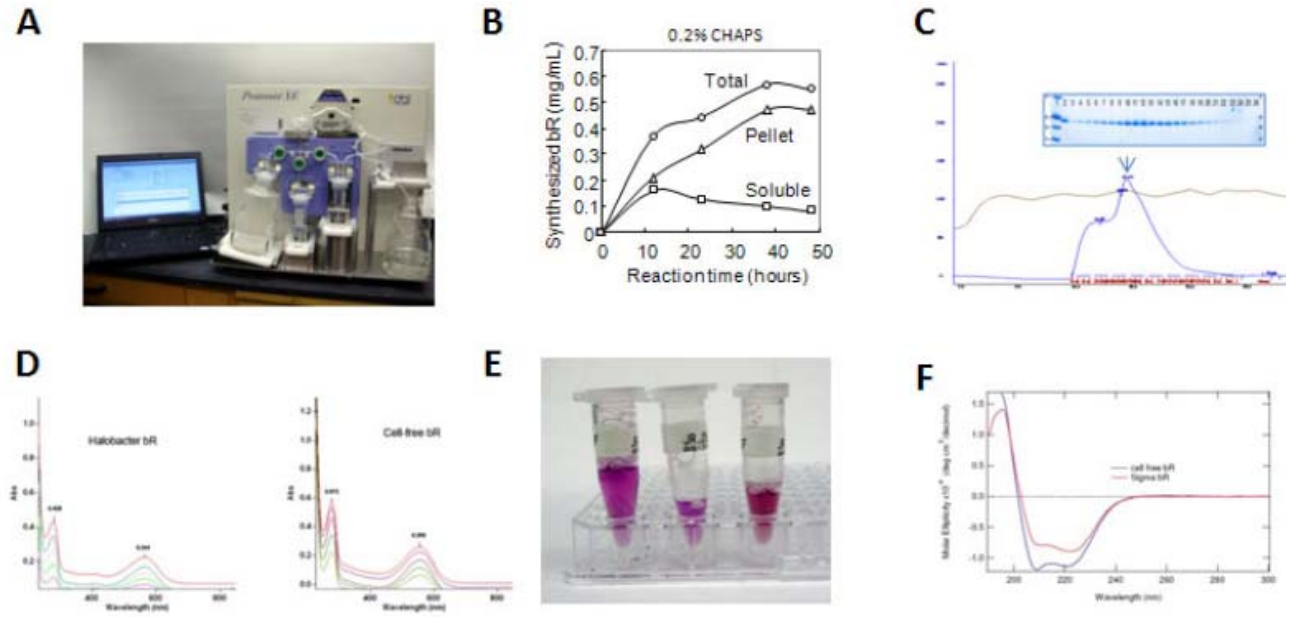

Summary

We demonstrated the efficacy of the Protemist XE (**Panel A**), a compact cell-free translation system capable of producing yields of membrane protein sufficient for NMR or crystallography. This tangential-flow dialysis system was used with optimized wheat germ extract and buffer conditions to synthesize the 7-TM *Halobacter* proton pump bacteriorhodopsin (bR) in the presence of 0.2% CHAPS and 0.1 mM all-*trans*-retinal (**Panel B**). The initial synthesized yield was 5 mg of seleno-methionine-labeled bR precipitate from a 10 mL reaction. Further adjustments of detergent conditions in both the reaction and feeding mixtures are expected to improve these yields. Greater than 95% bR was solubilized in 0.5% FC-12. This material was purified by IMAC and buffer exchanged into 0.05% DDM. The protein was gel-filtered in 5 mM MES, pH 5.5, 0.025% DDM, 100 mM NaCl, and 0.3 mM TCEP, producing a peak corresponding to a protein:detergent complex size of 135 kDa (**Panel C**), consistent with dimeric or trimeric bR in DDM micelles. 1.3 mg of deeply pigmented protein was purified with an optical purity (A_{280}/A_{555}) of 2.2 (**Panels D and E**). In comparison, the A_{280}/A_{565} ratio of *Halobacter* bR was 1.9. The protein was stable for weeks at 4°C in these buffer conditions. Circular Dichroism spectroscopy showed that the cell-free bR was strongly alpha-helical and spectrally equivalent to bR from *Halobacter* membranes (**Panel F**). Peak flattening evident in the *Halobacter* bR preparation was likely due to the presence of lipids, which were lacking in the detergent-synthesized cell-free preparation. Crystallization trials are underway for the cell-free bacteriorhodopsin using hanging drop, bicelle, and lipidic cubic phase methods.

Acquiring the Technology	Contact Brian Fox bgfox@biochem.wisc.edu.
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