

A Protocol for the Identification of Membrane Protein Interaction Networks

Chiann-Tso Lin¹, Kristin D. Victry¹, Diana J. Bigelow, Dale A. Pelletier², H. Steven Wiley¹ and Brian S. Hooker^{1,*}

¹Pacific Northwest National Laboratory, Richland, Washington, USA

²Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

*E-mail: brian.hooker@pnl.gov

Nearly one-third of all genes in various organisms encode membrane associated proteins that participate in numerous protein-protein interactions important to the processes of life. However, membrane protein interactions pose significant challenges due to the need to solubilize membranes without disrupting protein-protein interactions. Aiming at development of a protocol for the identification and characterization of membrane protein interactions, we have tested the feasibility of isolating and identifying several protein complexes in *Rhodospseudomonas palustris*. *R. palustris* is a purple, non-sulfur phototrophic bacterium commonly existing in soils and water. Subunits of several *R. palustris* complexes were independently cloned with both a 6-histidine tag and a V5 epitope tag at the protein C-terminal end and used as baits for isolation of endogenous complexes. Cells expressing individual tandem tag fused proteins were then cultured under illumination at 30°C under anaerobic conditions.

Cells solubilized with non-ionic or zwitterionic detergents were subjected to affinity “pull-down” experiments, using either Ni-NTA gels alone or a two-step tandem procedure, first with Ni-NTA, and then using a V5 epitope specific antibody. Tryptic digested peptide fragments of the pull-down products were then subjected to LC-MS/MS analysis.

Our results show that independent of the ATP synthase subunits selected (RPA0176 [β , 51 kD], RPA0844 [F_0 -b, 19.2 kD], RPA0177 [γ , 32 kD], or RPA0178 [F_1 - α , 55.17 kD]) as baits for pull-down experiments, the vast majority of the complex members were found in the eluted fractions from the Ni-NTA column. The electrophoretic pattern of proteins also confirms the ATP synthase complex similarity when using different baits. Further evidence for the feasibility of isolating the ATP synthase complex in the presence

of detergents has been solidified with the tandem pull-down using V5 antibody after the Ni-NTA step.

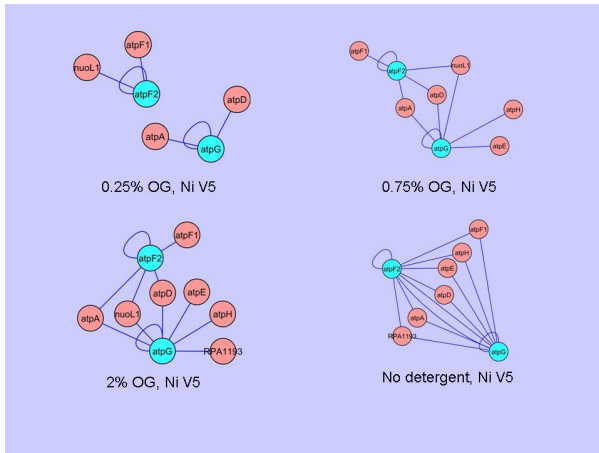


Figure 1: Results of the ATP synthase γ chain (atpG) and F₀-b chain (atpF2) pull-down experiments.

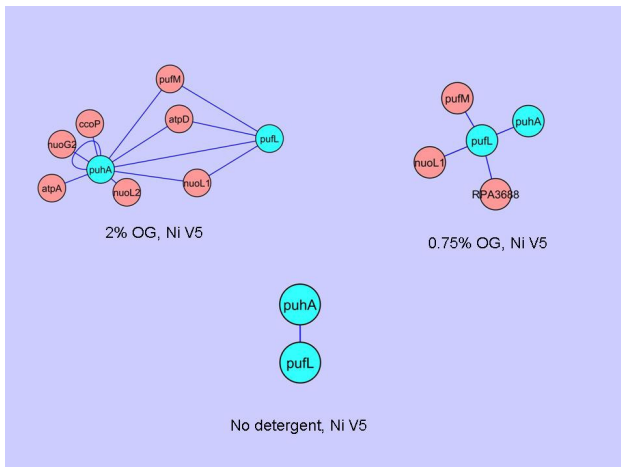


Figure 2: Results of the photosynthetic reaction center subunits L (pufL) and H (puhA) pull-down experiments.

In this case, the 0.25% OG trial was dropped and pull-downs were completed for 0.75% OG, 2% OG and no detergent conditions. Out of the 3 conditions tested, 2% OG performed the best, yielding 7 known prey proteins as well as the interaction between the two bait proteins.

These experiments demonstrate the efficacy of the tandem pull-down method in the presence of detergent for the isolation and characterization of protein complexes. Further

An example of a pull-down experiment for the ATP synthase is shown in Figure 1. In this instance, different detergent concentrations both above and below the critical micelle concentration for octyl- β -D-glucopyranoside (OG), at 1.0%, were employed in tandem Ni-NTA/V5

antibody pull-downs using the γ chain and the F₀-b chain as baits. Bait and prey proteins are shown as teal and pink circles, respectively. Out of the four conditions tested, 0.25% OG performed the worst, yielding just 4 known interactions between the two bait proteins. In contrast, the other conditions (0.75% OG, 2% OG and no detergent) performed comparably well, yielding 8-9 known prey proteins.

A similar experiment was completed using photosynthetic reaction center complex protein subunits L (pufL) and H (puhA) as shown in Figure 2. In this

data will be presented to compare the effectiveness of different detergent formulations as well as other pull-down conditions.