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## Analysis of Protein Complexes from *Rhodopseudomonas palustris* by Mass Spectrometry

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Protein complexes from *Rhodopseudomonas palustris* are analyzed by mass  
Novel Aspect: spectrometry at high throughput.

### Introduction

The bacterial species *Rhodopseudomonas palustris* is of interest both scientifically and practically because of its ability to produce hydrogen, degrade lignin monomers, and survive under a variety of conditions (aerobic/anaerobic, light/dark). As part of the Genomes to Life Center for Molecular and Cellular Systems, we are culturing *R. palustris* under a variety of growth conditions, and identifying the expressed proteins and protein complexes ("molecular machines") using mass spectrometry-based proteomics techniques.

### Methods

*R. palustris* clones are being produced that express a selected protein as a fusion with affinity tags, such as His6, GST, and the V5 epitope. Affinity purification of the tagged protein also co-isolates the proteins with which the tagged protein interacts. Analysis of these "pulldowns" by mass spectrometry provides identities of proteins that interact with the tagged protein. Both intact and trypsin-digested proteins are being analyzed, using LC-FTICR MS and LC-MS-MS on quadrupole ion trap instruments, respectively.

### Preliminary results

Several affinity-isolated molecular machines from *R. palustris* have been successfully analyzed. GroESL complex: After isolation of affinity-labeled GroEL2, the GroEL and GroES subunits from both *R. palustris* GroESL operons were identified by bottom-up LC-MS-MS. Nitrogenase complex: In three separate affinity isolations, each using a different affinity-labeled component of the nitrogenase complex (nifK, nifD, nifH), we demonstrated co-isolation and MS detection of the labeled component as well as the other expected components. ATP synthase complex: The beta subunit of ATP synthase complex was affinity labeled. Affinity isolation allowed MS detection of the labeled protein, plus the alpha, gamma, and delta subunits of ATP synthase. In addition to these complexes, LC-MS-MS analysis was performed for over 20 affinity-tagged fusion proteins expressed in *R. palustris*. Approximately 100 additional affinity-tagged targets are at various stages in the process (tagging, expression, purification, and analysis). Our goal is eventually to automate the entire process in high throughput, hands-off environment that will allow rapid and accurate characterization of the entire complement of protein complexes in a microbial species. This information will be a valuable resource for the biological community, for applications such as elucidation of biochemical pathways and eventually, a systems-level understanding of *R. palustris* and, other microbial species.

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